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FOREWORD

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S. STACHENKO, *President,*
Cane Sugar Refining
Research Project, Inc.

F. G. CARPENTER, *Research Leader,*
Cane Sugar Refining,
Agricultural Research Service

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GEL FILTRATION FOR DETERMINING EFFICIENCY OF COLOR REMOVAL IN PROCESSED RAWS

By N. H. Smith[1]

ABSTRACT

A gel filtration technique has been developed for measuring the ease of decolorizing individual colorants present in raw sugar. The method involves fractionation of samples through a column of Sephadex LH–20, and monitoring percent transmission at 420 nm. The data, obtained in graphical form, are analyzed with a Wang 600 programmable calculator. The ease of removal of the various colorants present is evaluated by comparing samples before and after the process steps of affination, defecation, decolorization by char, and crystallization. This technique shows promise as a means of relating variations in the decolorizability of raws to the composition of colorants as determined by gel filtration.

INTRODUCTION

The work presented here is part of a current study of the factors contributing to variability in the refinability of raw sugar, primarily with respect to color removal.

A considerable amount of information has been published concerning the large number of colorants present in cane sugar.[2] Several laboratories have examined the nature of these colorants and have estimated their ease of removal.[3] In a previous study,[4] an attempt was made to isolate individual colorants and then to evaluate their ease of removal from sugar with bone char. This approach was subject to several limitations, including the difficulty of isolating some fractions and the susceptibility of colorants to chemical change during the isolation procedures.

One of the colorant separation methods applied in this earlier work was fractionation with a Sephadex LH–20 column. This method has been developed into a sensitive liquid-chromatographic procedure. However, instead of attempting to isolate colorants and then test them for ease of removal from sugar liquors, samples were fractionated on Sephadex LH–20 before and after processing in order to evaluate changes in colorant composition of the sugars.

In addition to examining the effects of processing on amounts of various colorants present in the raws, a number of raws from different ports were compared. The purpose of this comparison was to determine whether the nature of the colorants present might vary in such a way as to account for the differences in ease of color removal.

MATERIALS AND METHODS

Samples of sugar before and after processing were collected. Whole raw sugar and the affined raw produced from the same whole raw were obtained from the refinery. Liquors were sampled at the clarifier station before and after clarification.

Tests on refinery char effluents indicated that insufficient color remained in the effluents for adequate instrument response in the subsequent fractionation. This, of course, would also be true for the crystallization of char-decolorized raw

[1] Senior research chemist, California and Hawaiian Sugar Co., Crockett, Calif. 94525.

[2] Farber, L., Carpenter, F. G., and McDonald, E. J. 1971. Separation of colorants from cane sugar. Int. Sugar J. 69: 323–328. Tu, C. C., and Onna, K. 1959. The non-sucrose constituents of Hawaiian raw cane sugar crystals. Proc. 10th Congr. Int. Sugar Cane Technol., pp. 291–304.

[3] Cookson, D., Parker, K. J., and Williams, J. C. 1971. Properties of sugar colorants removable by ion exchange. Proc. 1970 Tech. Sess. Cane Sugar Refining Res., pp. 104–113.

[4] Smith, N. H. 1969. Methods for separating sugar colorants. Proc. 1968 Tech. Sess. Cane Sugar Refining Res., pp. 105–116.

liquor. In order that darker samples for these tests might be obtained, affined raw sugar was methanol washed and the washings were concentrated. This liquor was processed in a laboratory char-column test, boiled in a laboratory pan.

The four sets of sugars, representing the four major color-removing steps in the refinery, were then fractionated in the LH–20 system in the following manner. A reservoir provides a source of solvent, which is an aqueous solution containing 10% methanol, 0.02 *N* sodium chloride, and 0.015 *N* ammonium hydroxide. This solution, at a pH of approximately 10.5, is pumped by an accurate piston pump at 120 ml/h through a 10-ft by 1-inch column packed with Sephadex LH–20, a chemically modified dextran derivative. This material provides a separation based on two factors: (1) the molecular size of the components to be separated, and (2) the differences in adsorption of the components on the Sephadex. The first fraction of colorant, the A fraction, is completely excluded by Sephadex LH–20 and contains colorants of high molecular weight. Because of absorption effects, the molecular weight of subsequent colorants cannot be assigned with certainty.

A 10-ml sampling loop is located between the pump and column. The sample contains 2 g of solids and 10% methanol. The addition of an excess of sodium chloride and ammonium hydroxide to the sample improved reproducibility, especially when the size of the sample was varied.

The column effluent is passed through a 1-cm path-length flow cell mounted in a Beckman DU spectrophotometer. The spectrophotometer is supplied with a line-operated power supply suitable for continuous operation and is modified for recorder output. In this study, only 420-nm measurements were made. The signal is recorded as the percent transmission (*T*) and is plotted as a function of time. Since the pump provides a constant flow rate, the *X*-axis is on a volume basis as well as a time basis.

The desirability of working with quantitative data led to the development of a simple computer program to transpose the graphical data into numerical results. Pairs of *X*,*Y* data from the graph are entered into the computer. The percent *T* from the *Y*-axis is converted to absorbance. Increments of area under the curve are calculated by treating each segment as a trapezoid. The sum of area increments between minimums gives the area of the fraction. From this is subtracted the area under the baseline. This area results in part from starting with the recorder pen offset from 100% and in part from instrumental drift, which produces a sloping baseline. The resulting area represents the quantity of color in the fraction. The total area represents the quantity of color in the total sample. It is numerically equal to the attenuation index of the sample times the grams of sample used. With 280-nm measurements, good agreement was found between the total area calculated from the graph and that obtained by direct color measurements. Although 280-nm measurements are nearly pH independent and much higher than other readings for a given size sample, they suffer from a major disadvantage—colorless components are also included in the measurements. With 420-nm measurements, the agreement between calculated area and measured product of attenuation index times grams of sample was poor, often by a factor of 2. This is attributed to the variation in pH of the column effluent being monitored.

Figure 1 shows two fractionation curves for the same sugar liquor. The two samples differed in the quantity of sample used and, in the upper example, by omission of the excess base normally used. The upper curve was monitored simultaneously for pH with color. The lower curve was run on a smaller sample, and refractive index was

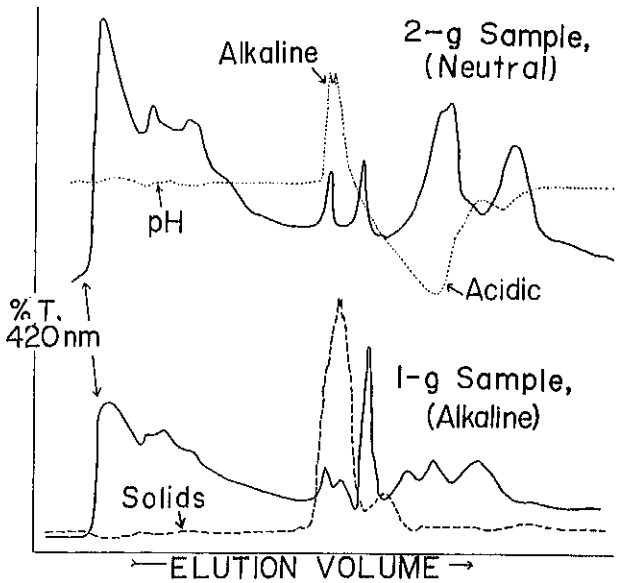


FIGURE 1.—Fractionations demonstrating separation of alkaline and acidic components (upper curves) and elution of solids (lower curves).

monitored simultaneously with color. In the upper curves, the separation of alkaline and acidic components is apparent. Since the pH of the eluant is variable, the color of pH-sensitive colorants will also vary uncontrollably. The highly alkaline peak coincides with the elution of sucrose and is probably sucrate. The acidic peak (a negative peak) coincides with a large color peak. Neither of these appears when the sample is made sufficiently alkaline. The acidic colorants are probably adsorbed by the Sephadex more strongly in the acid form and are eluted from the column sooner when more completely ionized by excess alkali.

The fractionation curve where the solids were eluted was erratic, even when sample size and alkalinity were controlled. Another factor contributing to the erratic curve was the effect of refractive index changes as sugar passed through the flow cell. Even a highly refined white sugar gave a 420-nm response in this region. Quantitative evaluation of the area under the curve was meaningless. Consequently, this part of the curve was not evaluated further. The remaining part of the fractionation curve was divided into eight fractions. The fractions are characterized by the presence of peaks, each representing the elution of a different colorant. (Electrophoretic studies have shown that the fractions, although generally showing one peak, are mixtures of two or more colorants.) The assignments of fractions are shown in figure 2. The ability to treat the eight fractions, lettered A through H, as individual areas, depends on the source of the sample. Usually it was found necessary to evaluate poorly separated colorant fractions in pairs.

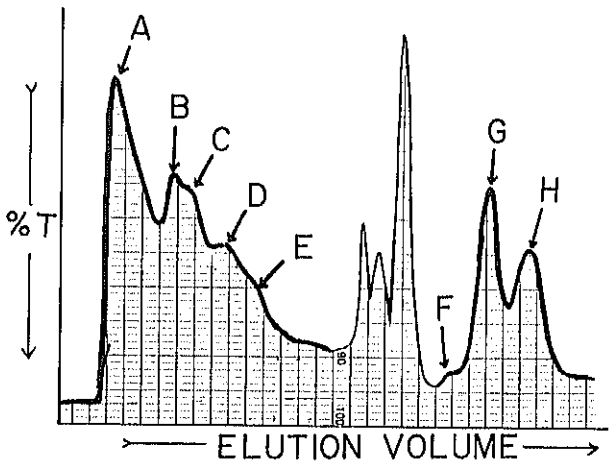


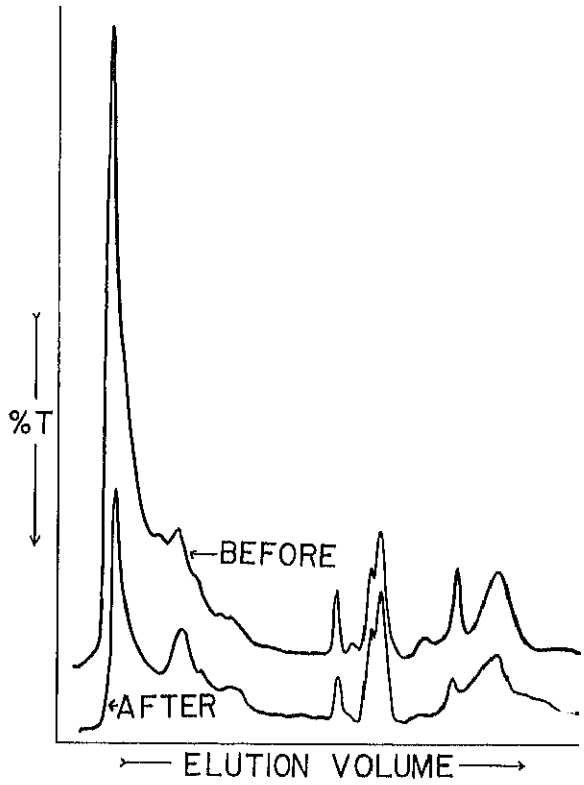
FIGURE 2.—Peak assignments in the Sephadex LH-20 fractionation.

RESULTS

Affination

The first decolorization step in refining raw sugar is affination. Figure 3 shows the color profile of whole raw sugar and the refinery-affined raw sugar it yielded. Qualitatively, there is a relatively large reduction in the first, or A, peak. The B and C fractions and similarly the D and E fractions are not distinguishable and are therefore treated as combined fractions for the quantitative evaluation. The H peak appears larger than the G peak in the affined sugar.

The calculated quantities of color in the various fractions are given in table 1. The qualitative evaluations from figure 3 are given a numerical basis. The largest reduction in color is found in fraction A, which has only 36% of its original color remaining in the affined sugar, whereas the H fraction contains the colorant the hardest to affine, with 93% remaining in the affined



affination.

FIGURE 3.—Fractionation of samples before and after

TABLE 1.—*Colorants in various fractions before and after affination*

[Colors measured at pH 10.5 and 420 nm]

Colorant fraction	Color in whole raw (color index units)	Color in affined raw	
		Color index units	Percent
A	2.79	1.01	36
BC	1.11	.73	66
DE	.25	.21	84
F	.08	.06	75
G	.21	.12	57
H	.55	.51	93
Total	4.99	2.64	53

sugar. These figures are extremes, to be compared with 53% of the total color remaining.

In considering the numerical data presented here, it should be kept in mind that the colors on which these results are based were measured at a pH of 10.5. Colorants which are pH-sensitive will appear to contribute more to the total color when measured at pH 10.5 than at pH 7.0; and, colorants which are harder to remove will increase the apparent percentage of total color remaining. Furthermore, the areas calculated are approximations, especially when a small peak follows a large peak. Part of the tailings of the large peak might be included in the small peak area.

Clarification

Inspection of the clarification curves in figure 4 shows that the first fraction (A) is larger before clarification than the second (BC), but that in the clarified liquor, fraction BC appears larger than fraction A. The data are presented quantitatively in table 2. The clarified liquor color, measured at pH 10.5, was 74% of the color before clarification. The colorant in fraction A was more easily removed than the average colorant, with only 58% remaining, while that of fraction BC was hardest to remove, with 86% remaining. Fraction H, hardest to remove by affination, was also hard to remove by clarification, with 85% remaining.

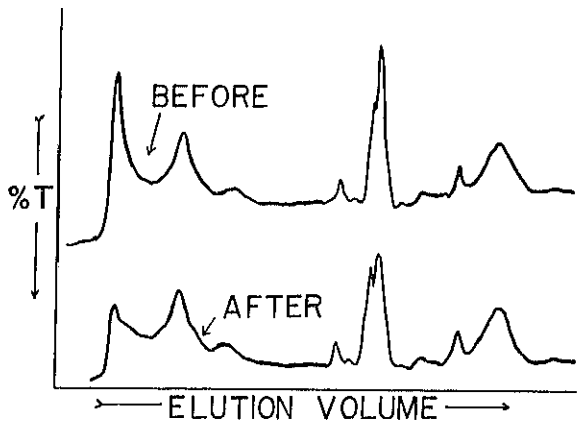


FIGURE 4.—Fractionation of samples before and after clarification.

TABLE 2.—*Colorants in various fractions before and after clarification*

[Colors measured at pH 10.5 and 420 nm]

Colorant fraction	Liquor color before clarification (color index units)	Liquor color after clarification	
		Color index units	Percent
A	0.77	0.45	58
BC	.70	.60	86
DE	.27	.20	74
F	.06	.04	67
G	.10	.08	80
H	.39	.33	85
Total	2.29	1.70	74

Decolorization by Bone Char

The extent of color removal obtainable in decolorization by bone char is greater than in other refining operations. To obtain measureably dark samples representing the early char effluents, a darker than usual on-liquor was used, as previously described. Char-column-test fractions were collected and fractionated separately on the LH–20 system. The curves obtained with on-liquor and with an early effluent are shown in figure 5. The decolorized sample represents an average No. 1 liquor, i.e., about 10% color remaining using conventional methods of measurement. A 3.77-fold expansion of the percent *T* scale was applied in recording the lower curve. Without an expansion of the percent *T* scale, analysis of relatively light-colored samples at 420 nm would be impracticable. Table 3 shows the quantitative results. Except for fraction FG, the area under each peak was evaluated separately. With an average of 8% color remaining in the combined fractions A through H, there are two fractions, B and D, that have twice that percentage remaining, whereas fraction H, hard to remove from sugar by affination or clarification, is easiest to remove with bone char.

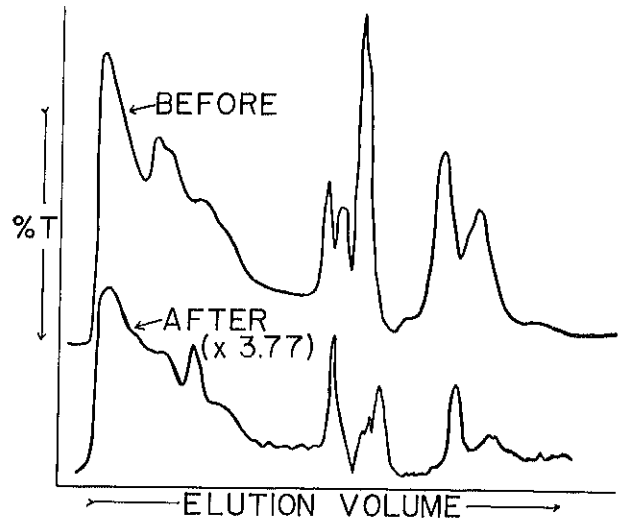


FIGURE 5.—Fractionation of samples before and after decolorization by bone char.

TABLE 3.—*Colorants in various fractions before and after decolorization by bone char (column test)*[1]

[Colors measured at pH 10.5 and 420 nm]

Colorant fraction	Liquor color before char (color index units)	Liquor color after char	
		Color index units	Percent
A	9.54	0.68	7
B	2.84	.47	17
C	4.43	.38	9
D	2.01	.34	17
E	4.56	.27	6
FG	3.77	.21	6
H	3.07	.16	5
Total	30.22	2.51	8

[1] Calculated for sample with 10% of total color remaining at pH 7.

Crystallization

Figure 6 shows curves representing the same liquor used as char test on-liquor before and after crystallization of the liquor. Although fraction BC is evaluated as a combined fraction, it has a double peak, with the first peak higher before crystallization and the second peak higher in the crystal. This means that fraction C is harder to remove by crystallization than fraction B.

In table 4 the crystal color is 23% of the liquor color taken as a whole. Fraction A contains the colorants most likely to boil into the crystal, with 34% remaining. Fraction DE contains the colorants most easily eliminated during crystallization, with only 9% remaining.

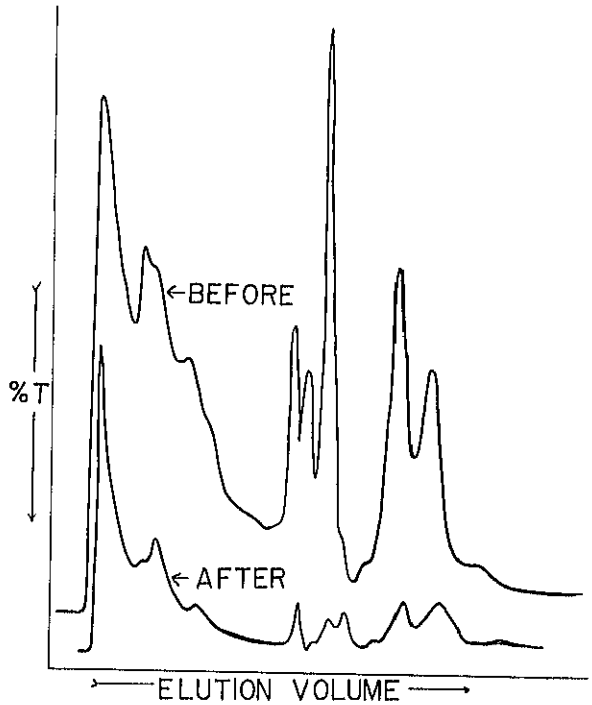


FIGURE 6.—Fractionation of samples before and after crystallization.

TABLE 4.—*Colorants in various fractions before and after crystallization (laboratory)*

[Colors measured at pH 10.5 and 420 nm]

Colorant fraction	Liquor color (color index units)	Crystal color	
		Color index units	Percent
A	9.54	3.26	34
BC	7.27	1.92	26
DE	6.57	.62	9
F	.31	.05	16
G	3.44	.38	11
H	3.07	.67	22
Total	30.20	6.90	23

Combined Refining Process

Since each phase of refining selectively removed different colorants from the sugar, how does the colorant composition affect overall color removal? This question requires two answers because the color of a granulated sugar depends on how much of each colorant is present in the raw sugar as well as on the ease of its removal. One answer can be obtained by combining the data in the tables already presented. The results

shown in table 5 indicate that in a whole raw sugar that might yield a crystallized white sugar with 0.7% of the original color remaining, colorants in fractions BC will most adversely affect the overall efficiency, with colorant H also harder to remove than the average. At the other extreme, colorants FG, A, and DE are relatively easy to remove.

TABLE 5.—*Combined effect of processing steps on colorant factors*

Colorant fraction	Percentage of color remaining after—			
	Affination	Clarification	Char	Crystallization
A	36	21	1.5	0.5
BC	66	57	7	1.8
DE	84	62	6	.5
FG	62	47	3	.3
H	93	79	4	.9
Total	53	39	3	.7

The second answer can be approached by looking at the variation in colorant composition among raws from different sources. As part of other studies in our laboratory, a survey has been made to screen raws from individual factory strikes, as well as from an occasional foreign raw. Whole raws were not examined. Only sugars which were affined and defecated on a laboratory scale were subjected to the LH–20 fractionation procedure. The color profile of these samples is shown in figure 7. Wide variations are apparent, with colorant A most significant in sugar P, and colorants BC and H more significant in sugars T and U. The numerical data are given in table 6. There appears to be little relation between the total color of the samples and the contribution by any given fraction. Fraction BC, which contains the most adverse colorants overall, is minimal in sugar P, accounting for only 0.11 out of a total color of 0.59 (about 19%), but predominates in sugar V, accounting for 1.04 out of a total of 2.69 color units, or 39%. Fraction A, which contains easily removed colorants, accounts for about half of the color of sugar P, but only about 8% of that in sugar T.

CONCLUSION

The techniques presented here show promise as a method of following the progress of removal

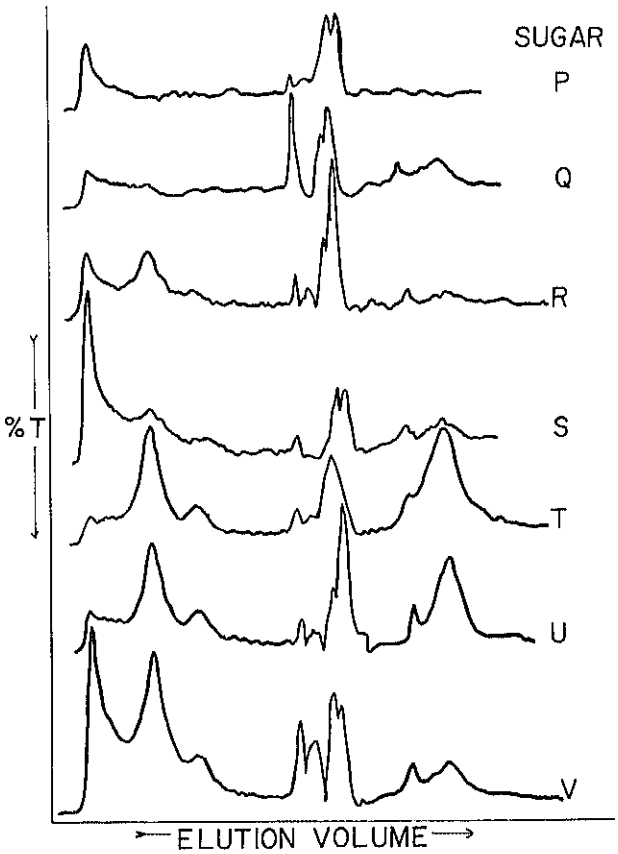


FIGURE 7.—Fractionation of samples of affined raws.

TABLE 6.—*Variations in colorant composition among raws*

[Color index units]

Colorant fraction	Color in each fraction of sugar—						
	P	Q	R	S	T	U	V
A	0.31	0.26	0.36	0.84	0.18	0.32	0.99
BC	.11	.21	.53	.45	.78	.81	1.04
DE	.07	.12	.23	.15	.24	.51	.32
FG	.08	.12	.08	.07	.12	.15	.12
H	.02	.27	.15	.15	.78	.81	.22
Total	.59	.98	1.35	1.66	2.10	2.60	2.69

of various colorants in the refining of raw sugar. There are significant differences in the ease with which colorants can be removed by any given refinery process. There are differences in how various processes affect a given colorant. And finally, there are variations among raw sugars with respect to the presence of easy-to-remove and hard-to-remove colorants.

DISCUSSION

M. K. FAVIELL (B.C. Sugar): Dr. Smith, do you have a class name for the BC colorants that you show on your graph? Also, have you any results on how carbon might affect the removal of this particular component or the component H?

N. H. SMITH: I have not made studies of other adsorbents. My general impression is that if the colorant is easily adsorbed by one adsorbent, it is easily adsorbed by another one. We find this to be generally true, using other adsorbents such as XAD–2, which I talked about previously, and carbon for total colorants in raw sugar. We can even include sugar as an adsorbent. Color easily adsorbed by char tends to be adsorbed by sugar crystallization. As for the class of colorants, I have no information as to their chemical nature. My inclination is to say that these are naturally occurring compounds. The BC comes out near the beginning and probably is of relatively high molecular weight, although not as high as the A component.

R. MOROZ (SuCrest Corporation): Most of your factionations showed large amounts in the A peak. I think you said these were high-molecular-weight colorants. What are the sources of these colorants?

N. H. SMITH: For peak A, again, we have not studied the chemistry. We know we can find them in the cane plant; even the cold-water extract of cane stalk or cane leaves show this high-molecular-weight fraction. One possibility is that these are products of enzymatic browning reactions. They may be from the polyphenols that react by enzymatic browning, and tend to polymerize. I do not know whether the high-molecular-weight colors are really in the plant before the juice is removed or not. I have the disadvantage of working with the sugar after it is crystallized as raw sugar.

C. C. CHOU (Amstar): Congratulations on this fine piece of work. Unfortunately the results are very complicated to explain. As you mention, in addition to the molecular-size effect and the adsorption effect in fractionation, there is also a partition effect if you use a solvent mixture such as water and methanol. My question is, what is the type of clarification? Is it phosphatation or carbonatation?

N. H. SMITH: The clarification is by phosphoric acid-lime.

W. L. REED (Revere): Would you comment on the comparison at the affination station of the color removal (you indicated about 47%) with what might be considered a standard removal from that raw sugar through a typical affination station, at the usual pH of 7 rather than your 10.5. I would usually expect to get 75% of the total color removed by affination. I realize, however, that you are at a different pH and so get a different effect.

N. H. SMITH: This was the sugar that was affined in the refinery. If we had measured it at pH 7, we would have found the usual 80% affination efficiency.

W. W. BINKLEY (New York Sugar Trade Laboratory): I would like to ask you to describe the individual colors of the fractions as brown or yellow.

N. H. SMITH: Peak A is gray brown. A very bright yellow band will show up if there are enough of the F, G, and H components. These later fractions tend to be the pH-sensitive and the fluorescent colorants.

W. W. BINKLEY: Does fraction A on electrophoresis break up into more than one color?

N. H. SMITH: On electrophoresis, fraction A streaks. The small bright spots of fluorescence are not visible in fraction A.

THE ACIDIC NATURE OF SUGAR COLORANTS

By Chung Chi Chou and A. E. Rizzuto[1]
(Presented by A. B. Rizzuto)

ABSTRACT

The acidic sugar colorants may be divided into (1) a strongly acidic carboxyl group, (2) a weakly acidic carboxyl group, (3) a phenolic-type hydroxyl group, and (4) a carbonyl group. These functional acidic groups can be identified by their reactions with bases of different strengths. Sugar colorants were first adsorbed in their acidic form; then, stepwise elution of the adsorbent with solutions of different bases fractionated the colorants into groups varied in acidity. A knowledge of the acidic nature of sugar colorants gives insight into the mechanism of color removal by adsorbents and other decolorizing agents.

INTRODUCTION

For many years, researchers in the sugar and food industries have tried to establish the nature and origin of sugar colorants. A brief review of the literature indicates that the colorants are generally classified into two groups: color which occurs naturally (plant pigments, for example) and color which is formed during sugar processing (caramel, melanoidins, for example). Each type may consist of a highly complex mixture of compounds. Various techniques have been used to separate the colorant mixture into as many individual components as possible. These attempts made it possible to characterize the nature of colorants and to study the behavior of various colorants in the refining process.

Tu and Onna (*10*)[2] used paper electrophoresis to study a colored sirup obtained from a methanol fraction derived from washed raw sugar. A negatively charged band, which finally resolved into five components under ultraviolet light, migrated toward the anode. A second band, brown in color, migrated toward the cathode. The brown band is thought to be the product of the reaction between amino acid and degradation products of sugars.

Gross (*6*) employed high-voltage electrophoresis to low-purity material, such as molasses, from various sources. A yellow and a brown fraction were obtained. Changes in the electrophoresis patterns were also studied as a function of two defecation methods. Carbonatation with larger than usual quantities of lime yielded additional brown color without removing many of the yellow colorants. Phosphatation effectively removed both brown and yellow colorants.

Farber and coworkers (*5*) studied the colorant constituents in cane sugar using solvent extraction and high-voltage paper electrophoresis as the principal methods of colorant separation. Since the identities of the colored substances were unknown, a system of spot numbers was devised to keep track of them. A total of 37 colored substances were reportedly found in cane sugar and process liquors. Their presence was followed throughout the various refining processes. The substances least removed by the present refining process were subjected to further study.

Gel filtration is commonly used to separate compounds by molecular weight. Smith (*8*) was able to separate the colorants of raw sugar and molasses into three main components on Sephadex G–10. The excluded materials were further subjected to gel filtration on Sephadex columns of increasing exclusion limits. The author then studied the spectral properties of each fraction at various pH's. It was found that the fraction of

[1] Research associate and assistant research director, Research and Development Division, Amstar Corp., 266 Kent Ave., Brooklyn, N.Y. 11211.

[2] Italic numbers in parentheses refer to items under "References" at the end of this paper.

high molecular weight was not sensitive to pH changes and was probably a melanoidine. The second fraction with a molecular weight close to that of sugar was also pH insensitive; the third fraction had a low molecular weight and high pH sensitivity. Abe and Iwashina (*1*) applied the same techniques to study the behavior of sugar colorants in the early stage of sugar refining. Sugar colorants in raw sugar, affined sugar, and carbonatated liquor were fractionated into four fractions on Sephadex G–10. The effect of various processes on the gel filtration color profiles were also examined. The affination process preferably removed the high-molecular-weight colorant from raw sugar. Carbonatation was effective in removing the high-molecular-weight colorant but not the fractions of low molecular weight. Cookson and coworkers (*4*) also employed gel filtration to obtain the molecular-size distribution of the standard synthetic and liquor colorants. The glucose colorant was shown to have the lowest molecular size, followed by the weakly basic, amphoteric, glycine colorant. The colorant formed in the presence of lysine gave a still higher molecular weight. The size of the liquor colorant was in the range between glycine and lysine colorants.

Column chromatography with ion-exchange resins is a relatively new technique for fractionation of sugar colorants. Using AV–16GS anion-exchange resin, Bugaenko and coworkers (*2*) fractionated the colorants in a molasses sample into four groups. First, a molasses was fed to the resin column. The colorants retained on the resin were fractionated by stepwise elution using water, 2% sodium chloride solution, 2% hydrochloric acid, and 2% sodium hydroxide. By comparing the spectral characteristics of each fraction with standard synthetic colorants, the authors assumed that the colorants eluted with water were derived from the caramelization of sucrose. Colorants desorbed by NaCl solution were identical to the colorant formed during the alkaline decomposition of reducing substances; colorants desorbed by HCl might be attributed to melanoidin, and those eluted by NaOH to humic substances. These authors stated that the reaction mechanism of the various agents on the desorption of the colorants from the resin was not known. Using the same technique, they also studied the selectivity of defecation processes toward various types of colorants (*3*).

Tsuchida and Komoto (*9*) described the application of DEAE-cellulose column chromatography to fractionate the nondialyzable colorants in the final molasses of a cane sugar refinery. The nondialyzable matter was applied to the column and eluted stepwise with 0.01 *N* acetic acid, 0.01 *N* formic acid, 0.05 *N* phosphoric acid, and 0.05 *N* phosphoric acid solutions containing various concentrations of sodium chloride. At least nine groups of colorants were obtained. Hydrolysis of these colorants gave at least four carbohydrates and several anionic compounds. The theoretical aspect of the desorption of the colorants from the DEAE-cellulose by these eluants was not discussed in the article. Parker and coworkers (*4, 7*) used DEAE-cellulose to study the net anionic charge of various colorants. The adsorbed colorants on the ion-exchange cellulose were eluted in the order of increasing net charge by a sodium chloride gradient. The distribution profiles gave the nature of colorants from various origins with respect to their net anionic charge. The amphoteric properties of colorants have also been studied by these researchers. Colorants with basic groups were adsorbed by Amberlite 200 cation exchange resin in H^+ form from methanol solution at pH 2. Colorants were then fractionated according to the base strength by elution with aqueous alkali.

This paper describes a method for fractionating colorants according to their acidic properties using Amberlite XAD–2. Changes in the color profiles are also obtained as a function of decolorizing systems.

METHODS AND MATERIALS

Column Chromatography on Amberlite XAD–2

Amberlite XAD–2 was packed in water in a 2.5- by 33-cm, organic-solvent-resistant Sephadex column equipped with two flow adaptors. The resin was washed with water, then with methanol, to remove impurities. The methanol was replaced by 1 mN hydrochloric acid. A desired volume of sugar liquor with a total color unit of about 60, adjusted with hydrochloric acid to pH=3, was pumped upward through the column at a flow rate of 1 ml/min. The column was then washed with acidic water at pH 3.

The colorants retained on the resin were fractionated by stepwise elution downward, using

0.1 *N* $NaHCO_3$, 0.1 *N* Na_2CO_3, 0.1 *N* NaOH, methanol, and 10 mN methanolic hydrochloric acid at a flow rate of 0.42 ml/min. A three-way solenoid valve was used to switch from one eluant to the other every 12 h. Column effluent was collected on a fraction collector; the period for each fraction was 30 min.

Since the fractionation of colorants by this technique is similar to an acid-base type reaction, the following experimental parameters should be kept constant: volume of each eluant, time of elution, color unit fed to columns, and the amount of XAD–2 used for each run. The results should then be analyzed on a relative basis.

The effluent color was measured at pH 7, and in some cases 9, by using ammonium acetate buffers in a 1:1 (sample-to-buffer) ratio. The measurement was done by the Technicon Auto-Analyzer at 420 nm. The column effluent was further fractionated on Sephadex G–10. Unless otherwise specified, sugar samples were washed with sucrose-saturated methanol.

Fractionation of Colorants by Ion-Exclusion Resin

Dowex 50WX4 in K+ form was packed in a 2.5-cm-diameter jacketed column. The resin height was about 2.5 m. The feed (molasses) was about 100 ml/run at 40 Brix. The column was eluted with distilled water at 180° F at a flow rate of 5 ml/min. A total of 50 fractions was collected with a fraction collector. Each fraction was about 28 ml. The effluent color was taken by the method described in the previous section.

Gel Filtration of Colorants by Sephadex G–10

The gel-filtration column was prepared according to the standard method described in the technical manual by Pharmacia Fine Chemicals, using 0.015 *N* ammonium hydroxide. Five ml of sample solution was carefully applied to the top of the 1.5- by 68-cm column. The gel column was then eluted with 0.015 *N* NH_4OH (pH 10.8) solution at a flow rate of 0.16 ml/min. The effluent was collected every 30 min in portions of about 5 ml. The color was measured as previously described.

In each run, the void volume of the gel bed and the elution volume of the colorants were determined. After each run, the column was washed with 0.05 *M* solium borate (pH=9.2) containing 0.02% sodium azide to remove residual color in the column. In the figures, when the color reading is off scale, the actual reading is stated adjacent to the peak.

RESULTS AND DISCUSSION

Column Chromatography Using Amberlite XAD–2

An appreciable number of sugar colorants are acidic. The functional groups of these colorants vary in acidity. In a solution of low pH, most of the acidic colorants are in nonionized forms. When these groups react with a base, the colorants are ionized, giving various anionic species. Since the ions are more heavily hydrated than their nonionized counterparts, ionic colorants are much more soluble than nonionic colorants. The degree of ionization also affects the hydrophilic-hydrophobic balance of the colorants. The hydrophilic property of an anionic colorant is considerably reduced in an acid solution. On the other hand, an amphoteric colorant may possess an ionic character in both acid and base solutions. This is because the basic group of the colorant has cationic function in acid solution, and the acidic group is ionized in aqueous alkaline solution to form an anionic colorant. The properties of a colorant depend greatly on its solubility, its hydrophilic-hydrophobic balance, and its ionic nature; hence there is a range of distribution of the acidic and basic groups of the colorant.

The acidic strength of the functional groups of various colorants can be differentiated by their reactions with bases of different strengths. Thus, a relatively strong acidic colorant is neutralized by sodium bicarbonate, sodium carbonate, and sodium hydroxide; a relatively weak acidic colorant is neutralized by sodium carbonate or a stronger base, such as sodium hydroxide, but not by sodium bicarbonate, etc. Accordingly, if sugar colorants are adsorbed in their acidic forms, stepwise elution of the absorbent with different bases neutralizes and thus ionizes the colorants of different acidic strengths. By this technique, the colorants can be separated into individual fractions with acidic groups of approximately the same acidity.

The hydrophobic adsorbent Amberlite XAD–2 serves this purpose. At low pH, most anionic colorants are retained by the resin through physical bonding, such as dipole-dipole interaction and

hydrophobic bonding. Then, elution of the resin with bases of increasing strengths, i.e., $NaHCO_3$, Na_2CO_3, and NaOH solutions, desorbs the colorants. The remaining colorants are washed out by methanol, then by methanolic hydrochloric acid solution. The colorants which are not adsorbed by the resin are presumably ionic under acidic conditions. This group of colorants probably includes (1) weakly basic colorants which exist in cationic form in acidic solution via their basic groups, (2) strongly basic amphoteric colorants such as zwitterionic colorants, and (3) strongly acidic colorants which dissociate to some extent even in acidic solution to give anionic species.

Three standard colorants were prepared according to the procedure described by Cookson (4), using 2% fructose solution. The fructose colorant is anionic, carrying no basic group in the polymer; the glycine and lysine colorants are amphoteric, having cationic function in acid solution.

Figure 1 is the color profile of the standard colorants according to their ionic nature and acidity, determined by the technique discussed under "Methods and Materials." As expected, the percentage of colorants which is not retained by Amberlite XAD–2 under acidic conditions increases from 6.5% for ionic colorant (fructose color) to 27% for weakly basic, amphoteric colorant (glycine color) and 37% for strongly basic, amphoteric colorant (lysine color). The basic groups of the amphoteric colorants are ionized at low pH to give cationic function. These ionic groups are highly solvated and resist adsorption by Amberlite XAD–2 at low pH.

As can be seen in figure 1, elution of the resin with various eluants fractionates the colorants into five groups differing in ionic nature. Fraction 1 (F–1), eluted by sodium bicarbonate, is presumably relatively strongly acidic. Fraction 2 (F–2), eluted by sodium carbonate, is less acidic. Fraction 3 (F–3) possesses a weakly acidic group which is neutralized only by a strongly basic sodium hydroxide.

The colorant obtained by methanol washing (F–4) is probably very weakly acidic or nonionic or both. In methanol solution, the adsorption is suppressed; thus, colorants which are too weakly acidic to be desorbed by the strong base NaOH were subsequently washed out by meth-

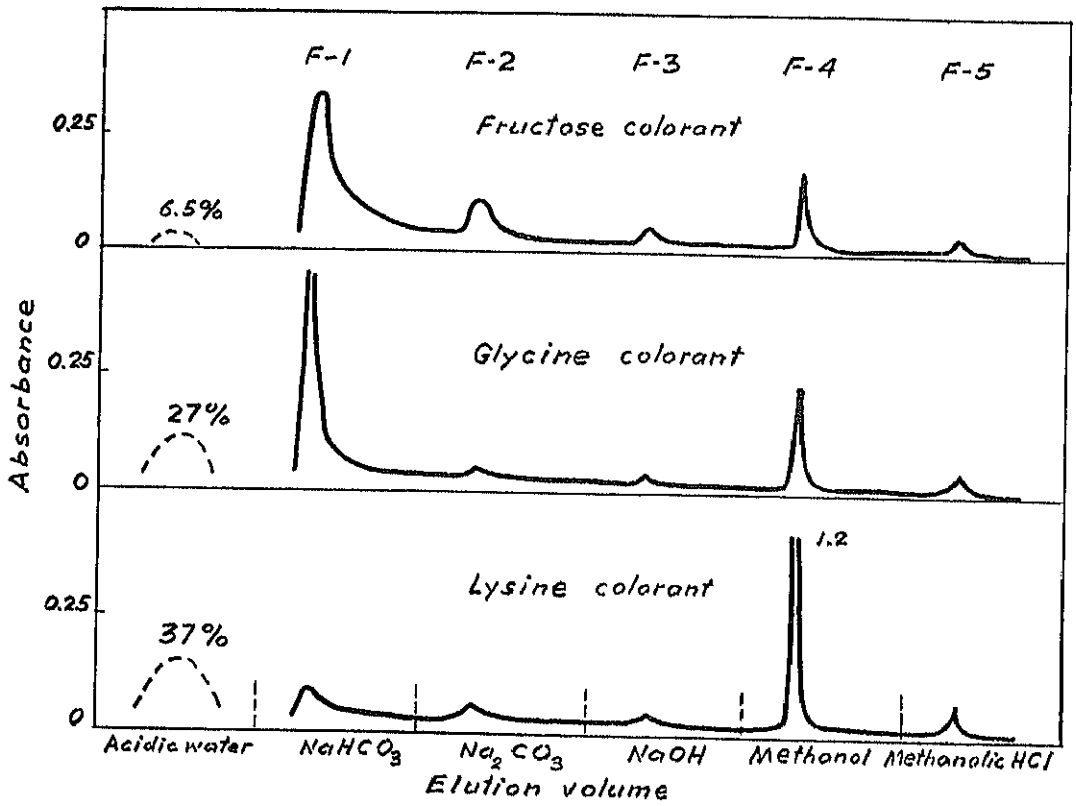


FIGURE 1.—Color profile of the standard colorants by their acidic nature, using Amberlite XAD–2.

anol. The colorants retained on the resin were eluted with methanolic hydrochloric acid. The mechanism by which the colorants were desorbed by acidic methanol might partly be attributable to the formation of cationic colorants via their basic groups. Less than 5% of those colorants which were adsorbed by the resin were not desorbed by the eluants used. The nature of these colorants, irreversibly retained because of their high affinity for Amberlite XAD–2 resin, remains to be studied. Comparison of the color profiles in figure 1 shows that lysine colorant has considerably less of the strongly acidic colorant (F–1) and more of the fraction eluted by methanol than fructose and glycine colorants.

The composition of the colorant as a function of acidity depends on the origin of the colorants. Figure 2 is the color profile, by acidity, of two raw sugars from different sources. The colorants not retained by XAD–2 are not shown. The distribution of colorants is considerably different for sugars A and B. Sugar A is composed of large amounts of relatively weak acidic colorants (Na_2CO_3 and NaOH fractions) and a methanol fraction.

The nature of colorants formed during sugar processing is governed by the availability of amino acid and other soluble nitrogen compounds. This is clearly indicated by the color profiles of the three standard colorants (fig. 1). In the presence of nitrogen compounds, a considerable number of amphoteric colorants were formed. These colorants were ionized at low pH and resisted adsorption by Amberlite XAD–2. Since nitrogenous compounds account for approximately half of the impurities in beet liquor, it is expected that the behavior of beet sugar colorants would be similar to that of those from glycine and lysine. The color profiles of a beet sugar liquor and a cane sugar liquor are shown in figure 3. At least 35% of the beet sugar colorants were not adsorbed by Amberlite XAD–2 at low pH, compared to 5% for cane sugar colorants.

The sensitivity of the technique was also tested, using tea and coffee colorants. The result is given in figure 4. It is observed that, whereas the tea colorants had a large amount of Na_2CO_3 fraction, the largest group in coffee colorants was in the methanolic fraction.

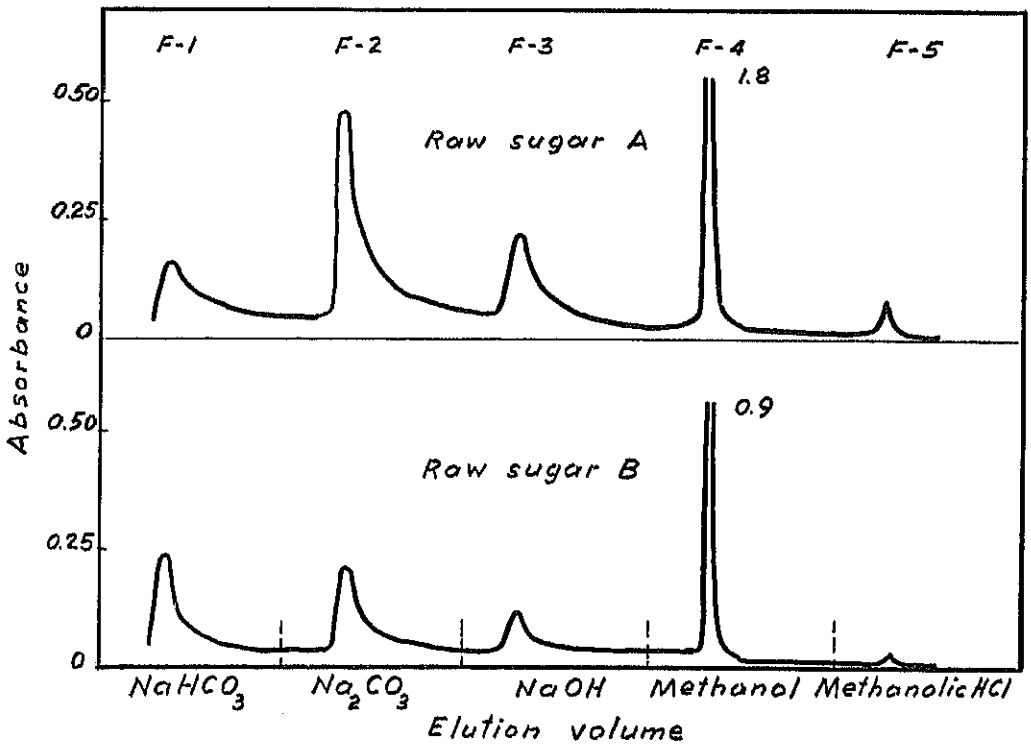


FIGURE 2.—Color profile of different raw sugars by their acidic nature, using Amberlite XAD–2.

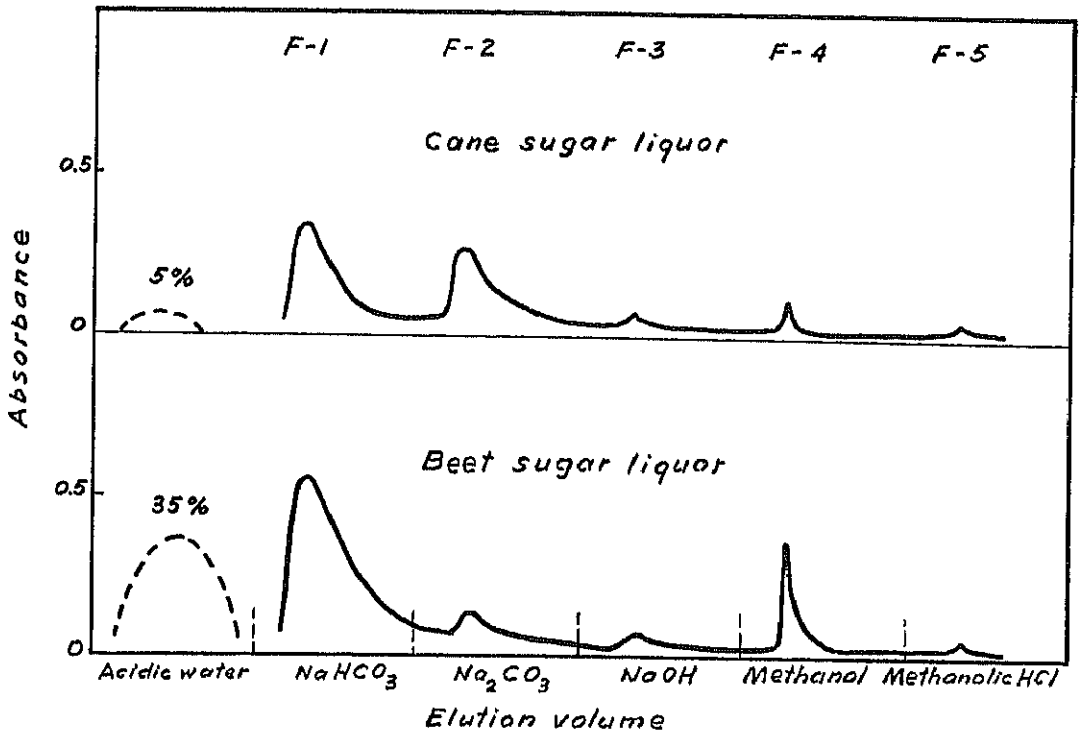


FIGURE 3.—Difference between cane and beet sugars as shown by their acidic nature, using Amberlite XAD–2.

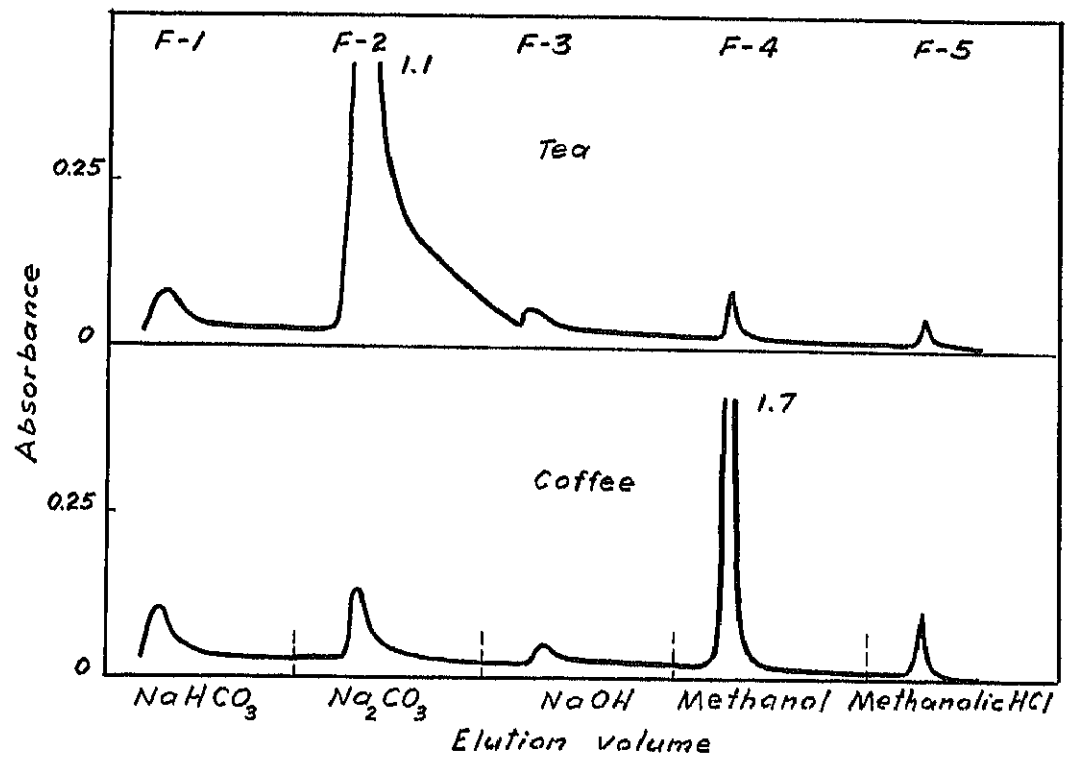


FIGURE 4.—Color profiles of tea and coffee.

Fractionation of Colorants by Ion-Exclusion Resin

The ion-exclusion process is based on the selective adsorptive capacity of certain ion-exchange resins toward compounds of different properties. For example, certain resins have a significantly higher adsorptive capacity, possibly through a complexing mechanism, for molecules of weakly ionized or nonionic compounds than for molecules of highly ionized compounds. Since acid colorants vary in their acidic strength and possess anionic properties in a base solution at a given pH, e.g., pH=7.5, the anionic character of the colorants is expected to increase from F–4 to F–1 fraction. Accordingly, when a sugar-colorant solution is fed into an ion-exclusion resin column, then eluted with water, the colorants emerge from the column in the order of decreasing ionic character, the highly ionized colorants excluded first.

Figure 5 shows the color profiles by ionic nature, using ion-exchange resin Dowex 50WX4 in K^+ form. Three peaks were obtained. The figure indicates that the majority of molasses colorants were highly anionic at pH=7.5. The highly ionic fraction (fractions 7–11) and the weakly ionic or nonionic fraction (fractions 35–45) were subjected to further fractionation by Amberlite XAD–2 at low pH, using various bases and methanol as previously described. The distribution patterns are shown in figure 6. It is not surprising to find that the highly ionic fraction (fractions 7–11) had a large quantity of strongly anionic colorants (F–1). The second fraction (fractions 35–45) contained mostly weakly anionic or nonionic colorants (F–2, F–3, and F–4). The distribution pattern of the feed material is also given in figure 6 for comparison.

Column Chromatography Using Amberlite XAD–2 at High pH

As discussed previously, the anionic nature of colorants depends on the pH of the solution. The higher the pH, the more the colorants will be ionized. At a low pH most acid colorants are in their acidic form. Since neutral molecules are generally less solvated, the adsorption of colorants is most effective in acidic solution. At high pH, e.g., pH=8, strongly acidic colorants exist in anionic form and have less tendency to be adsorbed. This is illustrated in figure 7. When the colorants were fed on to the Amberlite XAD–2 column at pH=3, five fractions were obtained by

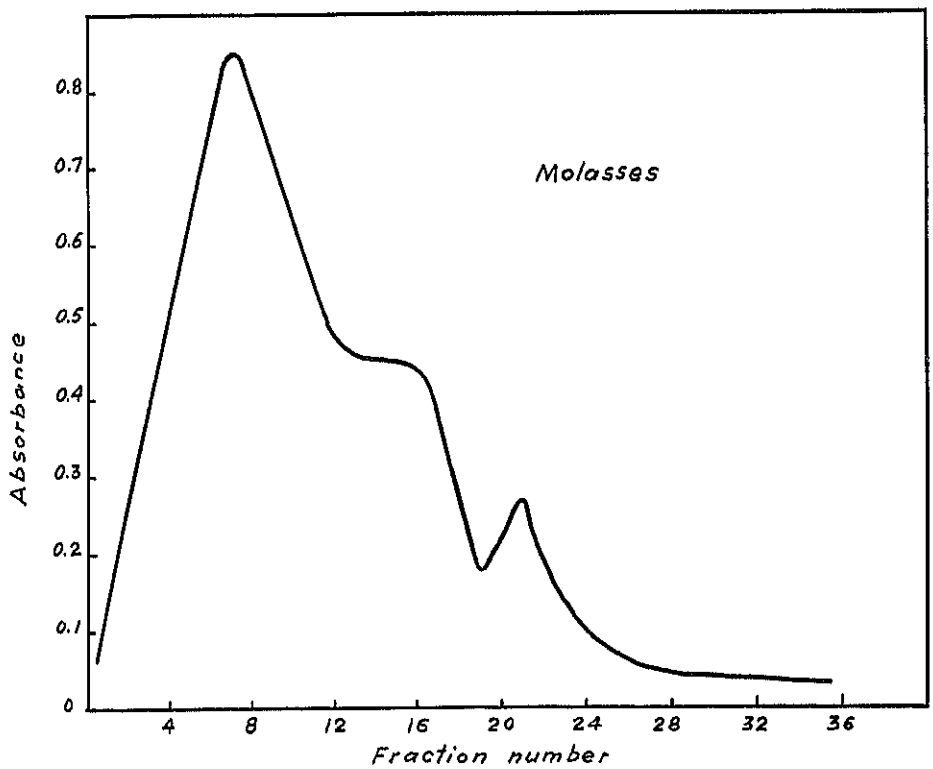


FIGURE 5.—Fractionation of molasses colorants by their ionic nature using Dowex 50X4 in K^+ form at 185° F.

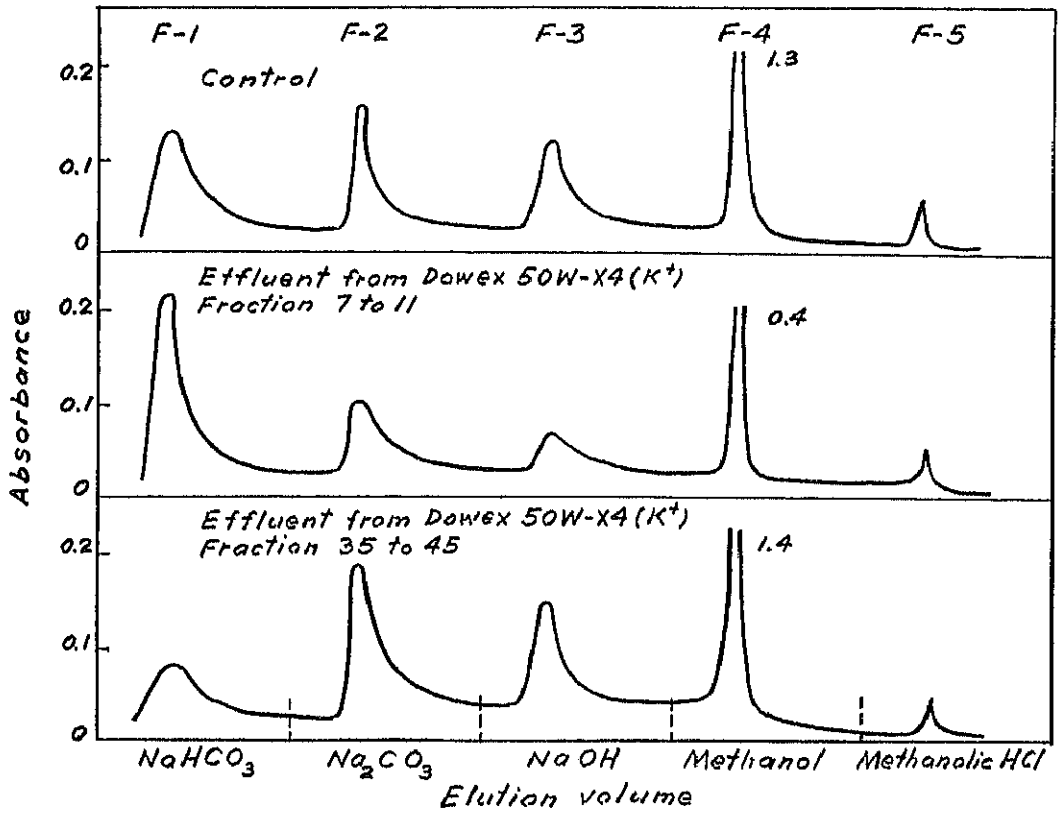


FIGURE 6.—Color profiles of different ionic fractions from figure 5.

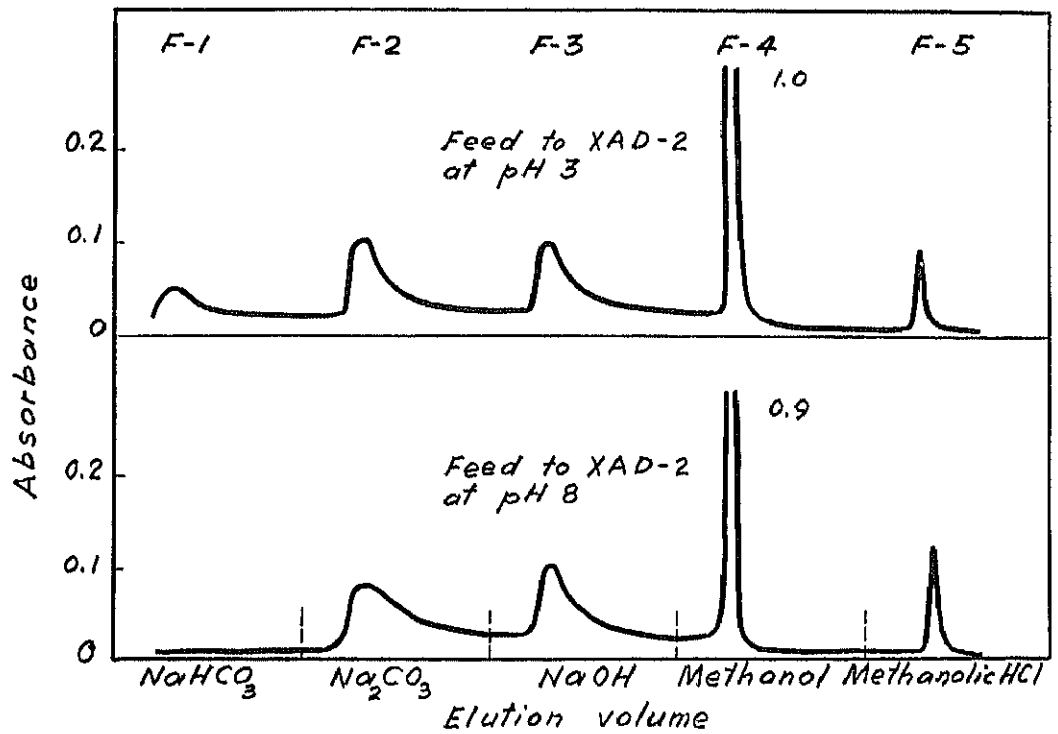


FIGURE 7.—Color profiles of the same washed sugar liquor at different pH.

elution with various bases and methanol. However, the strongly acidic peak was not observed when the feed was at pH=8, indicating that at this pH these colorants are ionized and, therefore, not adsorbed by the Amberlite XAD–2 resin.

Molecular Size Distribution of Colorants Using Sephadex G–10

The molecular weight and the distribution of colorants are important in the selecting of a suitable adsorbent or precipitant in a decolorization process. Generally, the efficiency of color removal by an adsorbent is a function of matrix pore size, tortuosity, and colorant molecular size, whereas the effective color precipitation process depends directly on the colorant's molecular size.

Gel filtration has previously been used to fractionate sugar colorants according to molecular weight. Although the adsorption of colorants by the gel matrix might significantly affect the result, the method would nevertheless be of value in indicating relative molecular-weight distribution.

The elution profiles of each colorant fraction (F–1 to F–4) on Sephadex G–10 are shown in figure 8 (Philippine Island raw sugar) and figure 9 (Taiwan raw sugar). The results show that the colorants with the same acidic strength were not necessarily homogeneous in molecular weight. Most fractions contained only traces of a yellow colorant with a low molecular weight, except fraction 2. The moderately strong acidic colorant (F–2) eluted by Na_2CO_3 consisted of about equal amounts of brown and yellow colorants. The brown colorant had a molecular weight above 700, and the yellow colorant, a molecular weight below 700. The pH sensitivity of each fraction is indicated in the figures by a solid line for pH 7 and a broken line for pH 9.

Color Profiles As a Function of Decolorizing Systems

Absorbents

Ion-exchange resin removes colorants mostly by the ion-exchange mechanism and to a lesser extent by matrix adsorption. When a sugar-colorant solution is brought into contact with an anion-exchange resin, anionic colorants are preferentially removed. A sugar solution was fed to an anion-exchange resin Bio-Rad AG–1X4 in Cl^- form and the effluent collected. The color profiles by acidity were obtained for the feed and the colored effluent (fig. 10). It is seen that the degree of decolorization by the ion exchanger increased with increasing acidity of the colorants, as anticipated.

Figure 11 shows the preferential adsorption of various colorants by bone char at different pH's as demonstrated by the changes in the color profiles before and after char decolorization. When

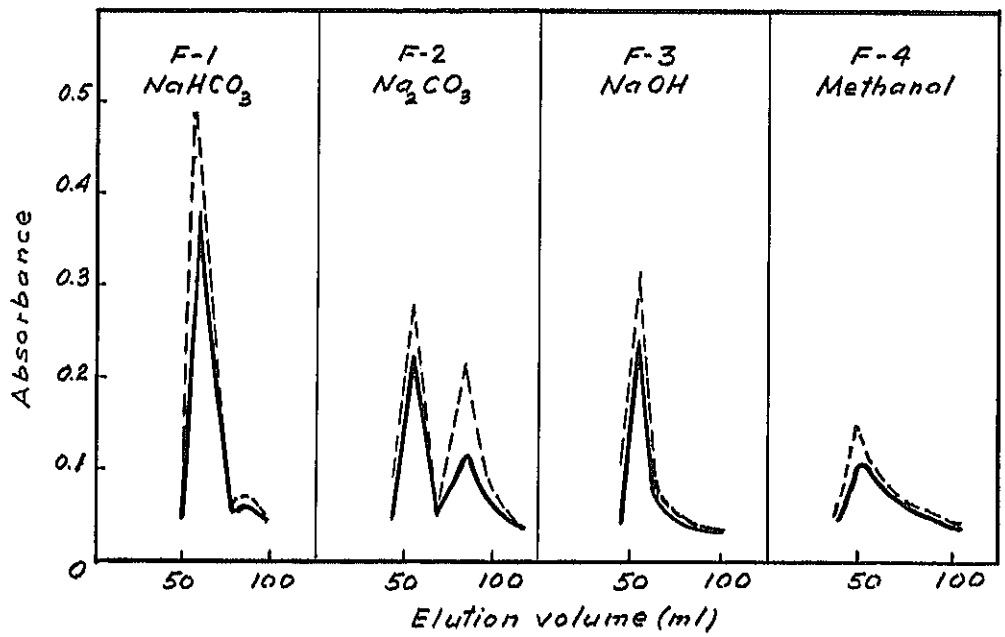


FIGURE 8.—Molecular size distribution by Sephadex G–10 of colorants of different acidic nature from raw sugar A.

the pH of the feed was 3, most of the acidic colorants were in their neutral form, and thus were removed by the char. At pH=7.5 an appreciable amount of strongly acidic colorants (F–1) was ionized and escaped adsorption by the char. It should be noted that most of the methanolic fraction (F–4) was removed at both pH levels. The nonionic or very weakly acidic colorant present

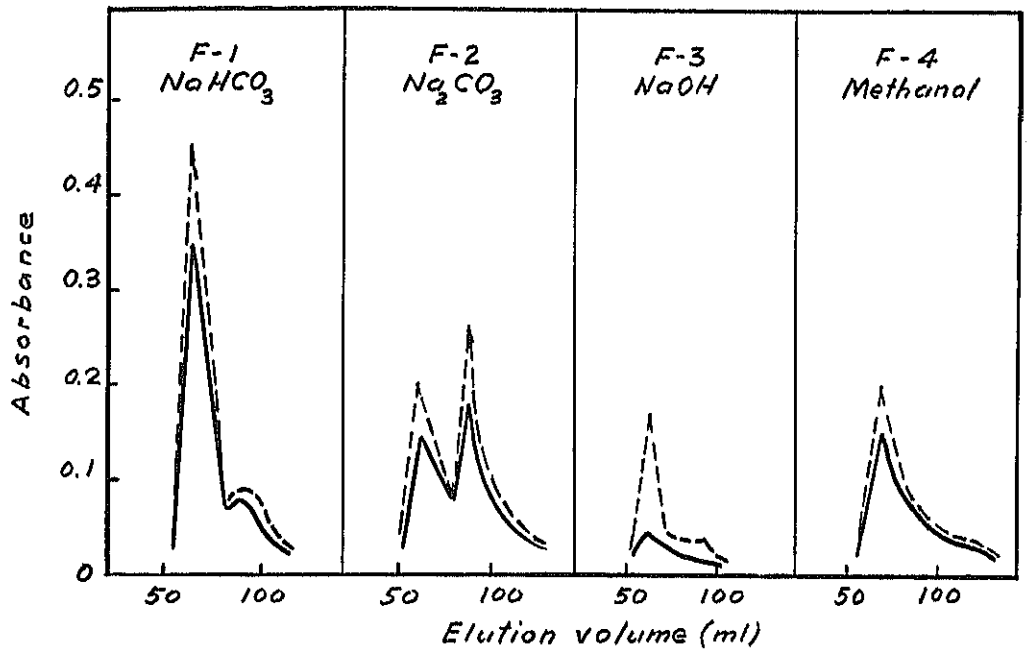


FIGURE 9.—Molecular size distribution by Sephadex G–10 of colorants of different acidic nature from raw sugar B.

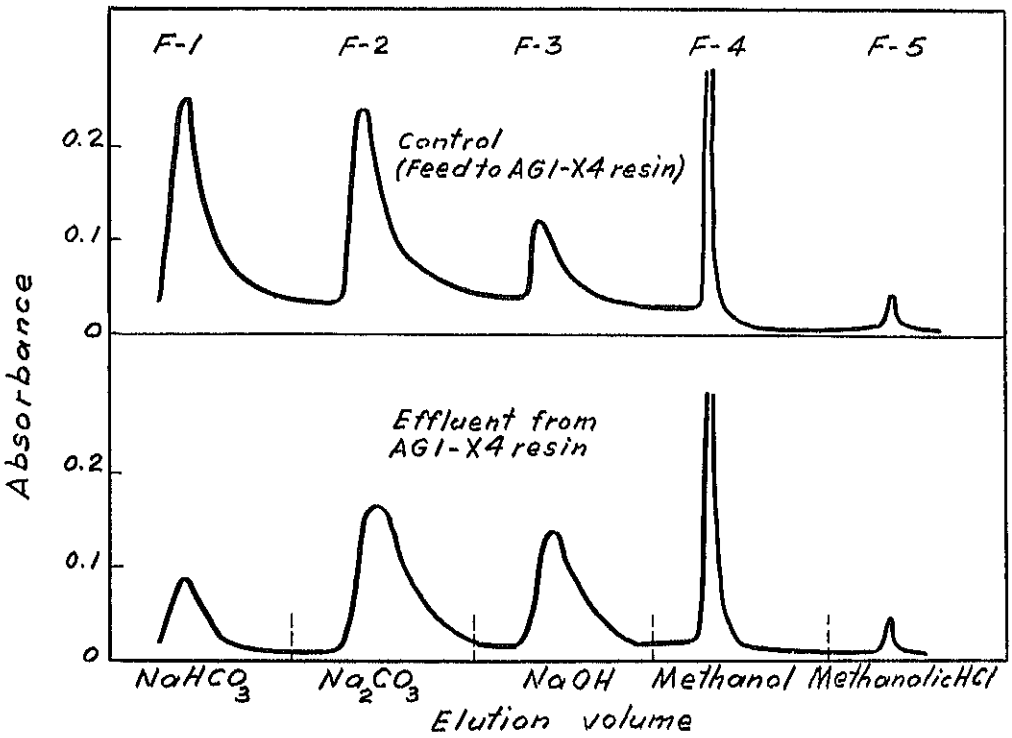


FIGURE 10.—Color profiles of a sugar liquor before and after anion exchange.

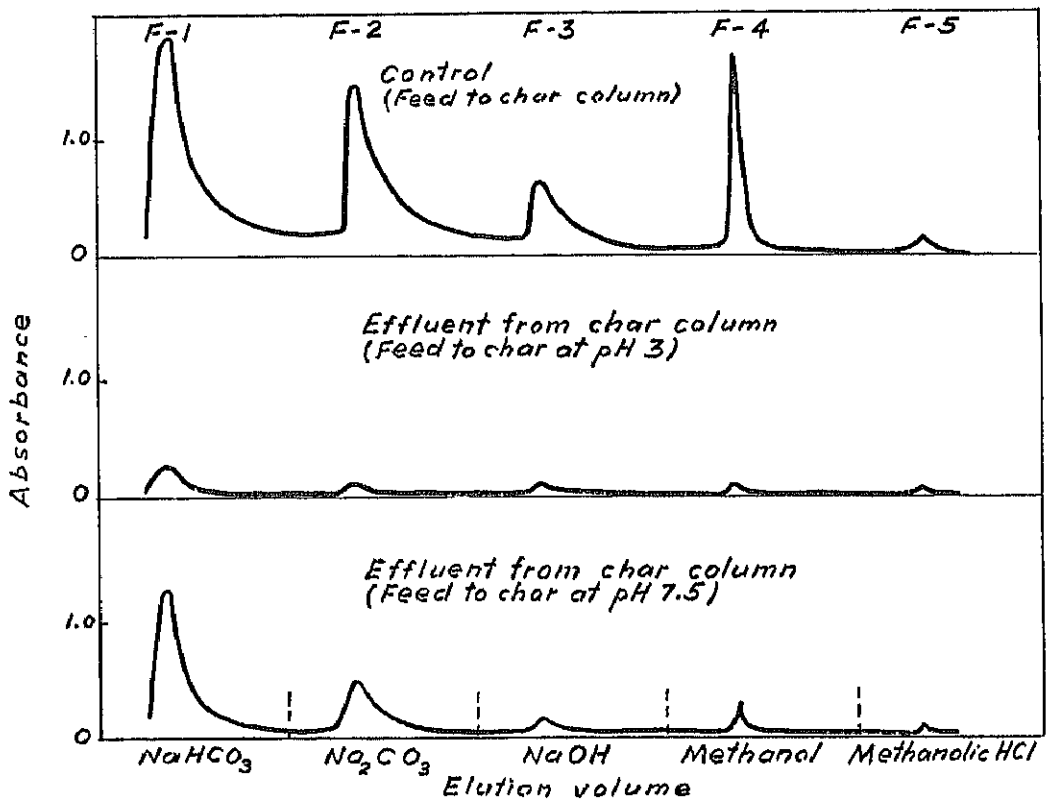


FIGURE 11.—Preferential adsorption of colorants by bone char.

in the methanolic fraction has more tendency to be adsorbed by char than the more strongly acidic colorant under the experimental condition. It should be mentioned that the color units fed to the XAD–2 columns were not comparable in each case because of experimental limitations.

Color Precipitation and Phosphatation

Long-chain cationic surfactants are known to form colorant-surfactant complexes through the interaction of the cationic charge of the surfactant and the anionic group of the colorants. Since the ionic centers are essential in the precipitation process, anionic colorant at a given pH is expected to be preferentially removed by the process. Figure 12 shows the color profiles before and after treatment of the liquor by phosphatation, with and without a cationic surfactant. The P_2O_5 added was 0.03% based on solids. The pH of the liquor after liming was 7.3. The ratio of the strongly anionic fraction (F–1) to the weakly anionic colorants (F–2) decreased considerably after the treatment, indicating the preferential removal of anionic colorants by the color precipitant and phosphatation. The ratios are 0.75, 0.33, and 0.28 for the control sample, and the samples treated with P_2O_5, and P_2O_5 plus cationic surfactant, respectively.

The selectivity of cationic surfactants toward colorants having similar acidity but different molecular sizes was also studied. A $NaHCO_3$ fraction and a Na_2CO_3 fraction were subjected to precipitation by a cationic surfactant (dimethyl dioctadecyl ammonium bromide). The molecular-weight distribution patterns of the colorants before and after the treatment were obtained (fig. 13). This preliminary work suggests that the colorants of high molecular weight are preferentially removed by the precipitant from the colorant mixture having similar acidity. Further study in this area is in progress.

Crystallization

To determine which types of colorants have less tendency to be occluded into a sugar crystal, a raw sugar was washed with a 60-Brix colorless sanding sugar solution to remove the "molasses" coating on the raw-sugar crystals. The washed raw sugar and the washings were subjected to fractionation according to their acidity. The col-

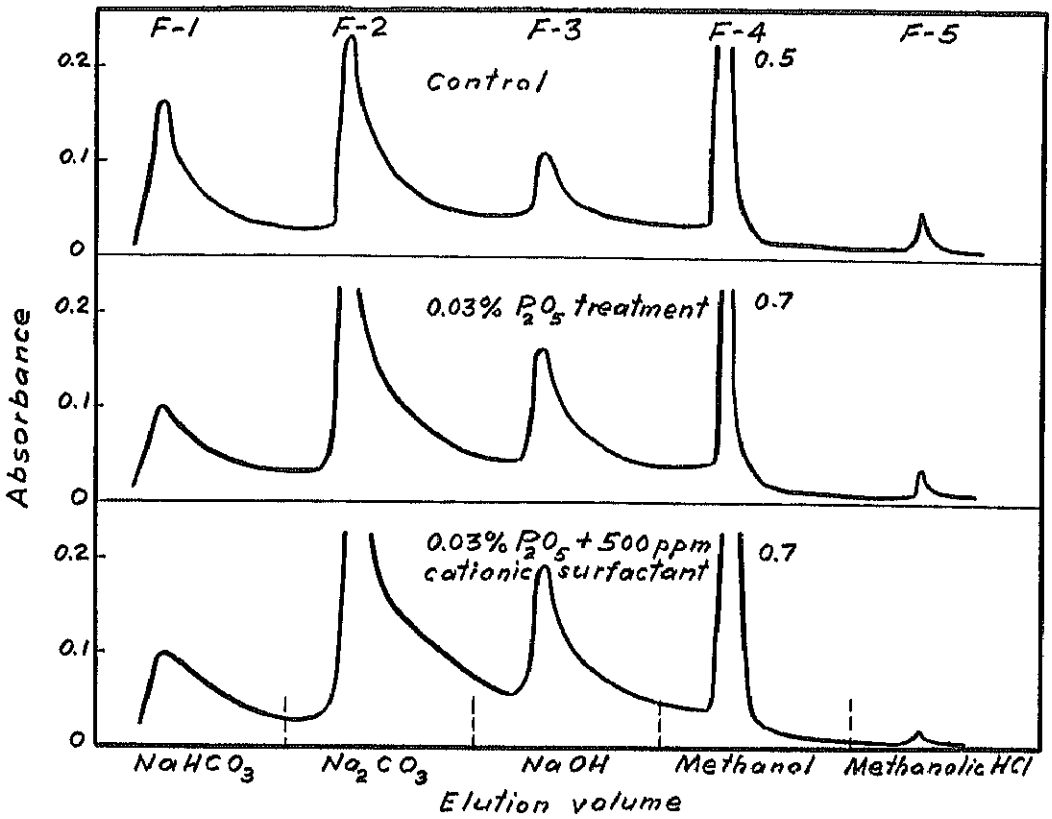


FIGURE 12.—Color profiles in phosphatation.

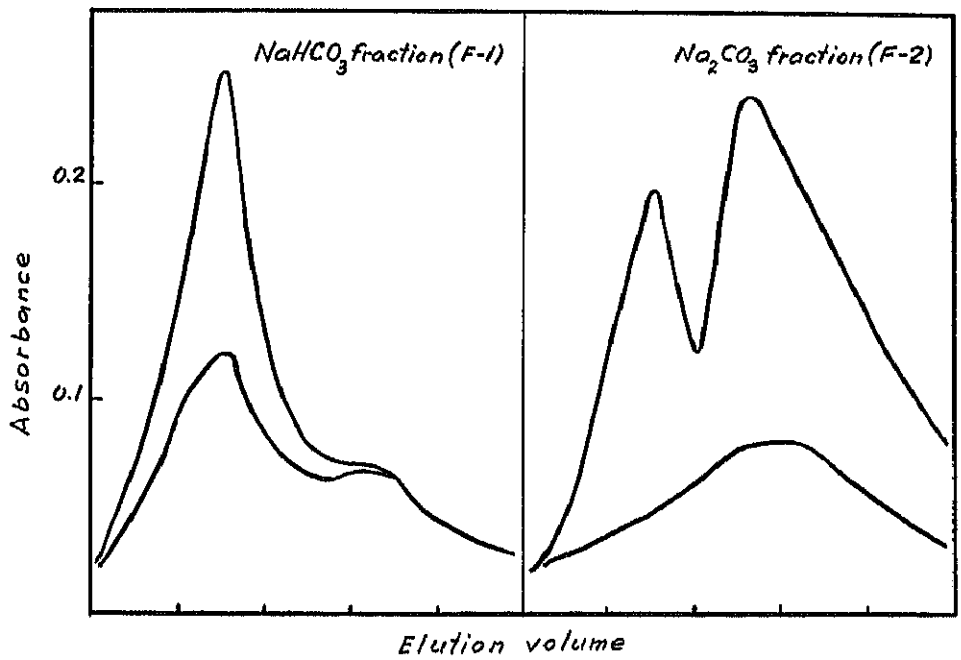


FIGURE 13.—Effect of cationic surfactant on molecular size distribution.

or profiles are given in figure 14. It is apparent that the crystal film contained significantly more strongly anionic colorant (F–1) than the crystal itself. The results suggest that highly ionic colorants are more apt to be excluded from the sugar crystal than weakly ionic or nonionic colorants, other parameters being equal.

Figure 15 gives the color profiles of two No. 4 granulated sugars after washing. The sugars were washed with a sucrose-saturated methanol to remove the colorants in the molasses film surrounding the sugar crystals. The fact that the color patterns of both No. 4 sugars had an F–1 to F–2 ratio of less than 1.0 seems to be in agreement with our previous findings for washed sugar crystals.

The foregoing observations might not be true if other parameters change. For example, the degree of colorant occlusion during a crystallization process might depend on the concentration of that particular colorant in the feed liquor. It is also conceivable that other interfering substances in feed liquor could have a significant effect on the selectivity of the colorant occlusion process. Further work in this area is anticipated.

CONCLUSION

Colorants with acidic groups can be separated from those with basic groups by selective adsorption, using Amberlite XAD–2 at low pH. The basic groups were ionized at low pH and thus resisted retention by the adsorbent. The colorants retained by the adsorbent were further fractionated into at least five fractions, using bases of different strengths and methanol solutions as eluants. This method serves to differentiate the acidic groups according to their acidity. These functional groups may include (1) a strongly acidic carboxyl group, (2) a weakly acidic carboxyl group, (3) a phenolic-type hydroxyl group, and (4) carbonyl group.

There are two major mechanisms of color removal: (1) adsorption, e.g., decolorization by carbon or adsorbent resin, and (2) ionic interaction, e.g., decolorization by anion-exchange resin or cationic surfactant. The efficiency of such a system is governed to a great extent by the anionic nature of the colorant, which in turn is determined by the degree of ionization of the acidic group.

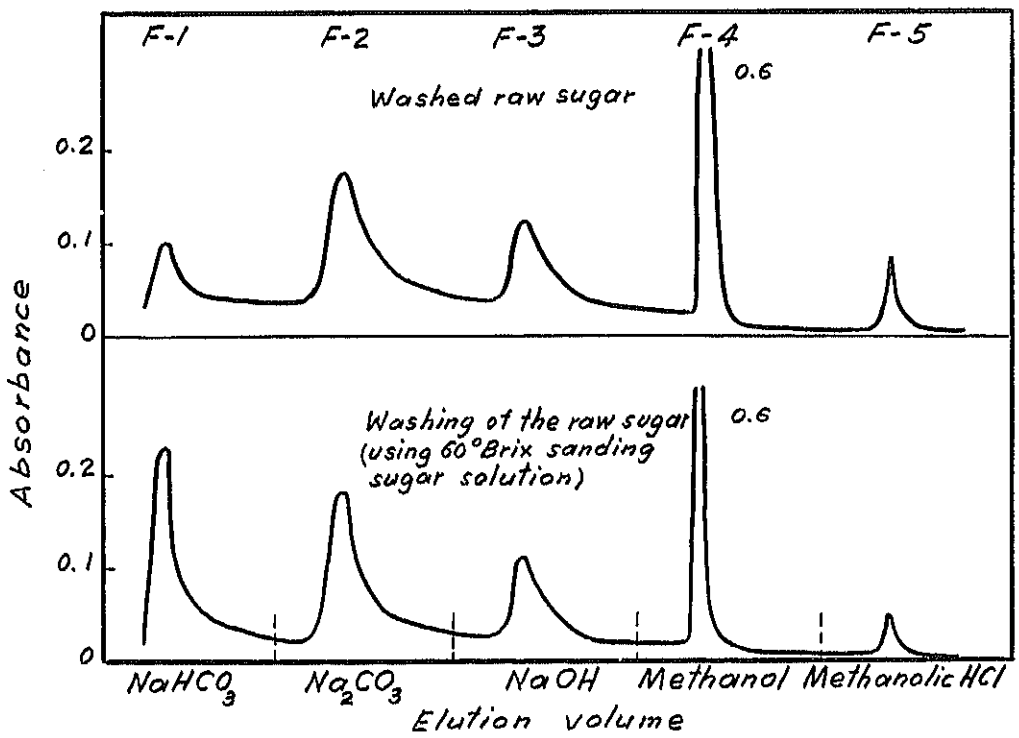


FIGURE 14.—Color profiles of a washed raw sugar and the washings.

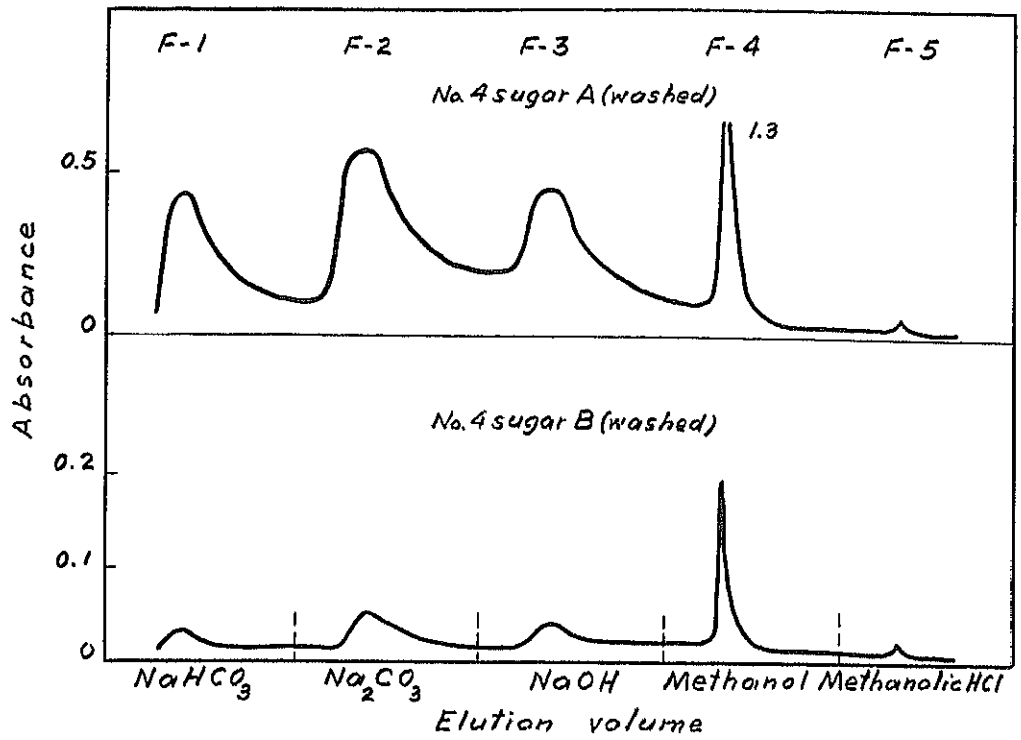


FIGURE 15.—Color profiles of No. 4 sugars.

It follows that the decolorization by cationic surfactant or anion-exchange resin is most effective when the colorants are in their anionic form. On the other hand, the adsorption by carbon adsorbents or adsorbent resins is most efficient when a colorant is in its nonionized form.

The effects of the interaction of the acidic and basic groups on the decolorization process are not considered in this presentation.

REFERENCES

(1) Abe, M., and Iwashina, S. 1970. Ion-exchange decolorization in cane sugar refining. Forecasting of the behaviour of sugar colorants during the first stage of refining. Proc. Res. Soc. Jap. Sugar Refineries' Technol. 22: 13–23.

(2) Bugaenko, I. F., Bulgakova, I. P., and Pavlov, I. I. 1969. Separation of coloring substances of sugar manufacture according to their adsorptive capacity. Sakhar Prom. 43: 6–9.

(3) ——— 1970. Separation of different groups of colouring substances during liming purification. Sakhar Prom. 44: 13–16.

(4) Cookson, D., Parker, K. J., and Williams, J. C. 1971. Properties of sugar colorants removable by ion-exchange resins. Proc. 1970 Tech. Sess. Cane Sugar Refining Res., pp. 104–113.

(5) Farber, L., Carpenter, F. G., and McDonald, E. J. 1971. Separation of colorants from cane sugar. Int. Sugar J. 73: 170–173.

(6) Gross, D. 1967. Fractionation and characterization of sugar colour by modern separation methods. Int. Sugar J. 69: 323–328.

(7) Parker, K. J., and Williams, J. C. 1969. The isolation and properties of sugar colorant. Proc. 1968 Tech. Sess. Cane Sugar Refining Res., pp. 117–128.

(8) Smith, N. H. 1967. Fractionation of sugar colorants with molecular sieves. Proc. 1966 Tech. Sess. Cane Sugar Refining Res., pp. 84–102.

(9) Tsuchida, H., and Komoto, M. 1970. Fractionation and some characterization of the non-dialyzable colorant in cane sugar refinery final molasses by DEAE-cellulose column chromatography. Proc. Res. Soc. Jap. Sugar Refineries' Technol. 22: 65–76.

(10) Tu, C. C., and Onna, K. 1959. The non-sucrose constituents of Hawaiian raw cane sugar crystals. Proc. 10th Cong. Int. Soc. Sugar Cane Technol., pp. 291–304.

ACKNOWLEDGMENTS

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DISCUSSION

L. E. MAHONEY (Revere): Would a change in adsorbent have any effect on the color profile?

A. B. RIZZUTO: Yes. This is conceivable because a change of adsorbent may affect the type and magnitude of the bonding between the adsorbate and the adsorbent.

G. W. MULLER (Kerr-McGee): I am surprised that you used a pH 3 treatment on bone char; would that not break down the char and confuse the results? Did you adsorb it on a column or in a batch?

A. B. RIZZUTO: We knew that pH 3 is not run in process. However, to verify our postulation, it was necessary to perform the experiment at low pH's.

G. W. MULLER: The carbon side of the bone char did more of the work than the hydroxyapatite. That may have disappeared at pH 3.

A. B. RIZZUTO: Possibly.

S. B. SMITH (Westvaco): I noted that the molecular-weight distribution for the yellow and brown colorants had a break at about 700 molecular weight. Do you have any idea what the approximate molecular size of those two groups would be, in angstrom units?

C. C. CHOU (Amstar): We know only that these colorants separate into a high-molecular-weight group (>700) and a low-molecular-weight group (<700). I believe Dr. N. H. Smith did some work in this area.[1] He subjected the first fraction to further fractionation by Sephadex of higher exclusion limit. He was able to separate it further, into three fractions instead of two.

N. H. SMITH (California and Hawaiian): In response to Dr. S. B. Smith's question, as I recall 50,000 molecular weight was the neighborhood in which the high-molecular-weight colorants hovered. There were some just below 50,000 and some above 50,000, when separated by Sephadex G–75 and G–200. I have a question about the use of methanol without prior dilution with the alkaline solvents. Do you know how effective methanol by itself would be in removing color without using the bicarbonate, carbonate, and sodium hydroxide?

C. C. CHOU: Methanol has a pH of about 9, and so it would wash off most of the colorants from F–1 to F–4. F–5 would be retained.

S. STACHENKO (Redpath): In figure 12 you show the color profile with two different types of treatments. One was the phosphate defecation alone, and the other was phosphate defecation combined with a 300-p/m addition of surfactant. Am I right to assume by the appearance of the curves, that the surfactant treatment did not appear to give any extra benefit to the phosphatation?

A. B. RIZZUTO: It did give some additional benefit that could be calculated, although the peak on the curve did not show it as well as we had hoped that it might.

W. W. BINKLEY (New York Sugar Trade Laboratory): In your model color preparations ou used glycine and lysine. Did you give any consideration to asparagine or aspartic acid, since these are the primary amino acids that we might expect to find in cane sugar? If you consider final molasses, the highest amino acid concentration is either aspartic or asparagine or both for cane; for beet, it is glutamic acid or glutamine. Glycine and lysine, I respectfully point out, are the amino acids present in the lowest concentration in cane final molasses. The other amino acid present in cane molasses in higher concentration is *beta*-aminobutyric acid which is very reactive. I assume you picked glycine and lysine because they are simple amino acids with markedly different properties.

A. B. RIZZUTO: We considered these other amino acids, but we picked lysine and glycine because we thought they would best exemplify the efficiency of the technique, and also because interpretation of the results would be less complicated.

W. W. BINKLEY: They will show that behavior because they are so different.

A. B. RIZZUTO: Yes. We will, however, in the future, experiment with the amino acids, such as asparagine and aspartic acid, that are commonly found in the process.

[1] Smith, N. H. 1966. Fractionation of sugar colorants with molecular sieves. Proc. 1966 Tech. Sess. Cane Sugar Refining Res., pp. 84–101.

PLANT PIGMENTS AS COLORANTS IN CANE SUGAR

By Leon Farber[1] and Frank G. Carpenter[2]

(Presented by Frank G. Carpenter)

ABSTRACT

The subject of plant pigments as colorants in cane sugar is reviewed and the work of the Southern Regional Research Center (Agricultural Research Service) summarized. Most of the compounds so far identified are derivatives of benzoic acid, cinnamic acid, coumarin, and flavone. Only the compounds in the last three categories are actually colored, but all are considered colorants in the broader sense of minor constituents.

INTRODUCTION

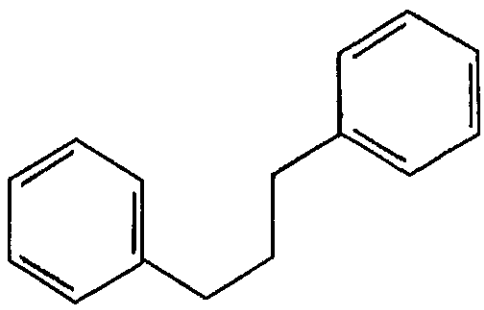
Although the word "colorant" as used in the sugar industry means specifically some substance that is not white, it can also mean color precursors that will soon become colored, and even undesirable minor impurities, colored or uncolored. In this paper we choose to use the word in its broader sense, because some of the pigments discussed are not colored and so are not true pigments; however, they chemically resemble colored pigments, undergo similar reactions, and have a similar deleterious effect on products using sugar.

One recognized source of colorants in cane sugar is the group of pigments from the cane plant. Gillett (*10*)[3] pointed out that the most obvious plant pigment, chlorophyll, is not soluble in water or sugar solutions, so there is no problem about its removal in processing. Xanthophyll and carotene, two other common plant pigments, are also insoluble and easily removed. However, the family of colorants called anthocyanins is present in cane and is soluble in water and so should enter the mill juice.

The anthocyanins are one of the oldest known classes of plant pigments; they were named as a class of compounds in 1835 and their chemistry was established by Willstätter and coworkers (*27*) in 1913. Synthesis and analytical procedures were established by Robinson and coworkers (*18*) in 1922. For several decades the anthocyanins were the only plant pigments studied much, and plant pigments and anthocyanins became almost synonymous. It was early established that anthocyanins easily hydrolyze into uncolored substances, or polymerize into insoluble substances (*27*). Also, all the anthocyanins are in the red to blue to purple color range which is not a common property of sugars. These facts are probably the source of the erroneous assumption that plant pigments are of no consequence as colorants in cane sugars.

The presence of anthocyanins in the cane has been confirmed by Parthasarathi and Vijayasaradhy (*17*), Seshadri (*20*), and Smith and Hall (*21*). Tu and Onna (*23*) reported anthocyanins in raw sugar, although more recently Smith and Hall (*21*) could not detect them past the initial cane-juice clarification stage.

Anthocyanins are only one subclass of a much larger class of flavonoid compounds having the common carbon skeleton $C_6—C_3—C_6$.



[1] 3048 Brighton 14th Street, Brooklyn, N. Y. 11235. Formerly with the Southern Regional Research Center, Agricultural Research Service, New Orleans, La.

[2] Research leader, Sugar Cane Refining, Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, New Orleans, La. 70179.

[3] Italic numbers in parentheses refer to items under "References" at the end of this paper.

Most of the naturally occurring members of the class have several substituent groups. The most common type has several —OH groups on the benzene rings, thus becoming polyphenols.

Polyphenols as a class easily react with iron or oxygen to form dark brown colors; their color is yellow and pH sensitive (indicator effect). These properties of polyphenols correspond well with the behavior of the sugar colorant and so polyphenols (sometimes shortened to phenolics) are readily interpreted as contributors to the sugar colorant. Gillett (*10*) observed that the polyphenols of cane juice derive mainly from tannin and anthocyanin; Parthasarathi and Vijayasaradhy (*17*) found the same. More recently, Bjerager and Bruniche-Olsen (*1*) described lignin as the chief source of polyphenols in cane juice.

McLaren (*15*), using solvent extraction, separated sugar colorant into three fractions which he identified as lignin-hemicellulose, polyphenols, and flavonoids. Gross (*12*), using electrophoresis, could separate the sugar colorant into many fractions and suspected that some of them were flavonoid pigments and phenolics.

Some hint about the individual compounds likely to be present in the sugar colorant can be obtained by surveying the compounds already known to be in similar plants. Van Sumere (*25*) lists the following compounds found in many grasses in order of decreasingly common occurrence: ferulic acid, coumarin, vanillic acid, *p*-hydroxybenzoic acid, *p*-hydroxycinnamic acid, syringic acid, umbelliferone, herniarin, aesculetin, chlorogenic acid, sinapic acid, scopoletin, *o*-hydroxycinnamic acid, and caffeic acid.

Stevens (*22*), using paper chromatography, found no phenolics in cane juice but found ferulic acid in the clarifier mud and *p*-coumaric acid and sinapic acid in the cane leaf. Caffeic acid could not be found. Tu and Onna (*23*), using a combination of solvent extraction, paper chromatography, and electrophoresis on raw sugar found cyanin, quercitrin, and several pigments of the catchin and chalcone classes that were not specifically identified. Ito and coworkers (*14*), using solvent extraction and thin-layer chromatography on molasses, identified vanillin, syringaldehyde, vanillic acid, and guaiacol. Hashizume and coworkers (*13*), using gas chromatography on cane molasses, identified anisole, phenetole, phenol, *m*-cresol, salicyclic acid, resorcinol, vanillic acid, syringic acid, *p*-coumaric acid, and vanillin. It is interesting that many of these same compounds are found in sugar beets and beet sugar (*2, 3, 15–18, 26*).

The main interest in maple sugar is flavor, which has been studied extensively. In this sugar a certain amount of flavor and color are desirable rather than objectionable. Underwood and Filipic (*24*) have identified one source of colorants as polyphenols derived from lignin from the maple tree.

Recent work in this laboratory (*7, 8*) showed that electrophoresis after the method of Gross (*11*) was the best separation method for the plant pigment type of sugar colorant. A spot numbering system was devised to keep track of the separated colorants, and it was shown that about one-third of the plant pigment type colorants eluded all refining steps and appeared in refined sugar. In another study one of the persistent spots which goes all the way to refined sugar was identified as chlorogenic acid (*4*). More recently nine additional spots were identified (*5, 6*). A total 21 spots have now been identified in this laboratory.

EXPERIMENTAL PROCEDURE

The sources of the colorant included all phases of processing: cane leaves, stalks, bagasse, cane juice, raw sugar, refined sugar, molasses, and many steps in between. In this initial work, all the details of the sources were not recorded. The cane came at different stages of maturity from Louisiana. Raw sugars came from all parts of the world. Refined sugar came from bone char refineries employing either phosphatation or kieselguhr filtration for clarification. All samples of any one type gave similar results.

The extracts from cane leaf, stalks, and bagasse were obtained by boiling the chopped-up material in water a few minutes and then filtering out the solids. The resulting highly colored broth was evaporated under vacuum and lyophilized. The portion of the dried material soluble in ethyl acetate was the source of colorants. The colorants from sugars, juices, sirups, and molasses were obtained by dissolving or diluting the sugar to about 10 to 20 Brix, then extracting by ethyl acetate. This procedure has been shown to be the most suitable for a broad spectrum of colorants (*7*).

Thin-layer chromatography was done on 8- by 8-inch plates with a layer 1 mm thick for pre-

parative work or a layer 0.35 mm thick for qualitative work. A large number of different solvent systems involving liquids with a wide range of polarity were used. These are given in table 1 and coded with a number. The adsorbents are given in table 2 and coded with a letter. The complete thin-layer chromatographic system can thus be defined by a number-letter code which can be deciphered with tables 1 and 2.

The standard conditions for electrophoresis were the same as those used previously (*5–8*), specifically: temperature, 15°± 1° C; paper, Whatman 3 MM, buffer 0.05 molar sodium tetraborate pH 9.2; voltage gradient, 100 V/cm; time, 45 min. Picric acid was used as the mobility standard and hydroxymethylfurfural as the zero marker. Mobilities given are in reference to these standards.

The procedure used to identify many of the constituents in sugar was a combination of thin-layer chromatography and high-voltage paper electrophoresis, and has been previously described (*5, 6*). In brief, it consisted of ethyl acetate extraction to separate the small amount of colorants from the great preponderance of sucrose. This was followed by thin-layer chromatography for the primary fractionation of the colorants because a recoverable amount of material could be obtained. This was followed by high-voltage paper electrophoresis as a monitor. High-voltage paper electrophoresis gives better separation, but being paper chromatography, it does not separate a recoverable amount of material.

In each case, the material from sugar was compared with an authentic known compound. The material was first run up the thin-layer plate. Fluorescence under ultraviolet light was used to find the position of the material on the plate. Nonfluorescent materials were detected with a pH indicator. Then both the material from sugar and the authentic compound were scraped off

TABLE 1.—*Volume composition of solvent systems for thin-layer chromatography*

Component	Solvent system																
	1	2	3	4	5	6	7	8	9	10	11	13	14	15	16	17	18
Methanol	2	3	3	1	..	..	..	..	1	1	..	..	2	1	..	..	..
Benzene	11	7	5	..	5	22	9	1	..	..	18	9	..	..	15	..	..
Acetic acid	1	1	1	2	1	1	..	..	..	..	1	..	..	..	..	..	..
Dioxane	..	..	..	..	..	6	..	..	..	..	..	..	..	..	..	..	..
Formic acid	..	..	..	..	..	..	4	..	..	..	..	..	..	..	1	..	1
Ethyl acetate	..	..	..	..	..	..	5	1	..	..	1	..	..	..	5	..	..
Chloroform	..	..	..	..	..	..	..	..	5	19	..	..	..	..	..	5	..
Acetone	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..
Water	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..
Methyl ethyl ketone	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3	..	..
Toluene	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	8	5
Ethyl formate	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	7	4

Component	19	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Toluene	5	..	..	..	5	..	..	..	..	10	..	..	..	2	..	..	..
Chloroform	..	5	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..
Acetone	..	..	..	..	..	..	..	..	5	..	..	..	..	..	..	..	..
Ethyl formate	8	..	..	..	4	..	..	..	..	10	..	13	..	3	..	..	..
Formic acid	6	1	1	..	1	..	..	..	..	3	..	1	..	3	..	..	..
Ethyl acetate	..	4	5	5	..	..	..	1	..	..	26	..	..	..	..	..	..
Methyl ethyl ketone	..	..	3	3	..	1	..	..	..	..	..	..	..	..	..	..	..
Water	..	..	1	..	..	..	..	..	1	..	7	..	13	..	1	2	1
Skelly B	..	..	..	..	..	..	..	2	..	..	..	..	..	..	..	..	..
Carbon tetrachloride	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..
n-Butanol	..	..	..	..	..	..	..	..	4	..	..	..	3	..	..	..	..
t-Butanol	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Isopropanol	..	..	..	..	..	..	..	..	..	..	14	..	..	..	..	3	1
Ethyl alcohol	..	..	..	..	..	..	..	..	..	..	..	..	3	..	6	..	..
Benzyl alcohol	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3
2,4-Pentanedione	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..

TABLE 2.—*Adsorbents for thin-layer chromatography*

Code	Adsorbent
A	Silica gel (Mallinckrodt).
B	Silica gel G (Mallinckrodt).
C	Silica gel GF7 (Mallinckrodt).
D	MN polyamide-DC11 (Machery Nagel).
E	Cellulose MN300 (Machery Nagel).
F	Avicel (Brinkmann).

the plate, redissolved, and run on electrophoresis. It was not until the match was obtained on electrophoresis that the test was considered positive. This was repeated at least three times with different thin-layer systems before a positive identification was claimed. In some cases when there was poor resolution, two thin-layer separations were run in series before electrophoresis.

Some compounds moved very little in electrophoresis, rendering it a poor method for identification. In these cases, gas chromatography was substituted for high-voltage paper electrophoresis as a method of monitoring the thin-layer chromatographic separation. It was necessary to silylate the compounds. The lyophilized and dried sample was dissolved in anhydrous pyridine and silylated by adding hexamethyldisilazane and trimethylchlorosilane. A Hewlett-Packard chromatograph model 5750 was employed. The column used was 1.5% SE 30 (silicon gum rubber) on Chromosorb P, 6 ft long, with a 1/8-inch outside diameter. An alternate column was 10% OV–I.

RESULTS

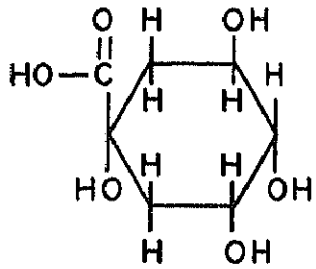
The results obtained for 21 compounds are summarized in table 3. The mobility relative to the front, R_f, is given for each of the thin-layer chromatographic systems used and also the electrophoretic mobility relative to the standards. The spot numbers used in previous work in this laboratory are given for each compound in order to correlate this work with previous reports (*5–8*).

All of these materials were found in the cane plant materials, and many persisted in varying amounts through various stages of sugar processing, even to refined sugar. The approximate extent of the persistence of these materials through the processing is also given in the table.

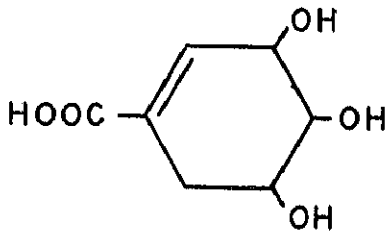
Table 4 lists the compounds in the order in which they appear in electrophoresis. Also given are the visible color and the fluorescent color upon excitation by light of 253- and 365-nm wavelengths. The negative numbers are for compounds that move backward relative to the starting point on the paper. There is a gap between spot Nos. 3 and 9 where no identifications have yet been made. Quinic and shikimic acids are not fluorescent and so are omitted from this table. Fluorescent spot No. 10 was previously studied (*8*) in this laboratory. The present identification as rutin substantially agrees with the previous conclusion that this material was a flavone of the apigenin series. Only the point of substitution of the glycoside proved to be different from that proposed in the earlier work.

DISCUSSION

Every compound listed contains a ring structure. The simplest structure is in quinic acid which has a saturated ring. This compound is common to many plants.



Splitting one water out of quinic acid gives shikimic acid,



This compound is considered to be involved in the biosynthesis of aromatics. All of the other compounds are aromatic.

Seven of the compounds are benzoic acid derivatives or their corresponding aldehydes.

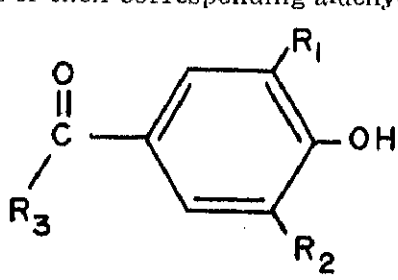


TABLE 3.—*Summary of identifying data for cane pigments*

Compound	Found in—	Electrophoresis		Thin-layer chromatography		References
		Spot No.	Mobility	System	R_f	
p-Hydroxybenzoic acid	Cane leaf	22.1	1.42	6B	0.39	4–6
				1B	.46	
				18B	.41	
3, 4-Dihydroxybenzoic acid	do	24.9	1.60	8B	.53	
				1B	.44	
				24B	.57	
4-Hydroxy-3-methoxybenzoic acid or vanillic acid	do	21.1	1.28	6B	.42	4–6, 13, 14, 26
				1B	.57	
				18B	.42	
4-Hydroxy-3, 5-dimethoxybenzoic acid or syringic acid	Raw sugar	17	1.07	6B	.49	4–6, 13, 19
				18B	.40	
				5B	.39	
p-Hydroxycinnamic acid or *p*-coumaric acid	Refined sugar	20	1.13	6A	.37	4–6, 13, 22
		21	1.18	1A	.53	
				17A	.43	
3, 4-Dihydroxycinnamic acid or caffeic acid	Raw sugar	22	1.34	1A	.27	4–6
		23	1.46	6A	.18	
				4A	.81	
4-Hydroxy-3-methoxycinnamic acid or ferulic acid	Refined sugar	14	.97	6A	.54	4–6, 22
		15.6	1.08	1A	.69	
				17A	.38	
4-Hydroxy-3, 4-dimethoxycinnamic acid or sinapic acid	do	12	.73	17A	.34	4–6, 22
		13	.92	18A	.42	
				6A	.41	
				1A	.50	
Chlorogenic acid	do	15	1.00	1A	.10	4–6
				3A	.60	
				4A	.77	
Quinic acid	Raw sugar			34F	.30	
				35F	.60	
				36F	.20	
Shikimic acid	do			34F	.50	
				35F	.70	
				36F	.40	
4-Hydroxy-3-methoxybenzaldehyde or vanillin	Cane Leaf	14.9	.96	11B	.28	13, 14
				26C	.21	
				13C	.45	
Coumarin	do	—5	.03	13C	.68	
				14D	.55	
				27C	.48	
7-Hydroxycoumarin or umbelliferone	Raw sugar	11.5	.82	22B	.96	4–6
				23B	.73	
				2B	.75	
				19B	.68	
Esculin	Cane Leaf	9	.60	22B	.43	
				33B	.45	
				2B	.60	

TABLE 3.—*Summary of identifying data for cane pigments*—Continued

Compound	Found in—	Electrophoresis		Thin-layer chromatography		References
		Spot No.	Mobility	System	R_f	
Coniferin	do	−3	.14	22B	.55	
				28B	.79	
				29B	.33	
				30B	.45	
Kaempferol	Raw sugar	1	.25	21G	.56	4–6
				18G	.41	
				7G	.60	
Quercetin	Cane rind	3	.34	15D	.07	
				17C	.46	
				16C	.50	
Rutin	Cane Leaf	10	.64	22B	.37	
				31C	.24	
				32D	.64	
p-Hydroxybenzaldehyde	do	17.1	1.10	10C	.47	
				11C	.16	
				13C	.38	
3, 4-Dihydroxybenzaldehyde	do	13.9	.90	9C	.51	
				25C	.62	
				8C	.51	

TABLE 4.—*Fluorescent cane constituents*

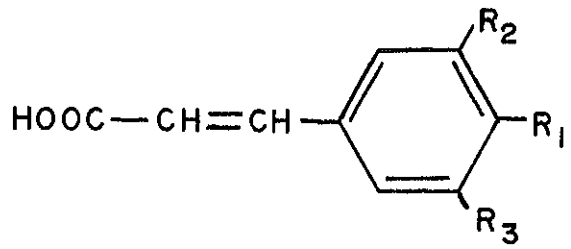
Compound	Spot No.	Fluorescence color, when excited by—		Visible color	Mobility[1]
		356 nm	253 nm		
Coumarin	−5	Blue	Blue	White	0.03
Coniferin	−3	Blue after standing	Black-blue	do	.14
Kaempferol	1	Yellow	Yellow	Yellow	.25
Quercitin	3	do	do	do	.34
Esculin	9	Blue	Blue	White	.60
Rutin	10	Yellow	Yellow	Yellow	.64
7-Hydroxycoumarin	11.5	Very bright blue	Bright blue	White?	.82
4-Hydroxy-3, 5-dimethoxycinnamic acid (*cis* and *trans* isomers).	12.1	Blue	Blue	Off white	.73
	13				.92
3, 4-Dihydroxybenzaldehyde	13.9	do	do	Light yellow	.90
4-Hydroxy-3-methoxycinnamic acid (*cis* and *trans* isomers).	14	do	do	*Cis*—yellow oil	.97
	15.6			*Trans*—white solid	1.08
4-Hydroxy-3-methoxybenzaldehyde	14.9	do	do	Off white	.96
Chlorogenic acid	15	do	do		1.00
4-Hydroxy-3, 5-dimethoxybenzoic acid	17	do	Blue then black	do	1.07
p-Hydroxybenzaldehyde	17.1	Black	Black	do	1.10
p-Hydroxycinnamic acid (*cis* and *trans* isomers).	20	Blue	Blue	Yellow	1.13
	21				1.18
4-Hydroxy-3-methoxybenzoic acid	21.1		Black	White	1.28
3, 4-dihydroxycinnamic acid (*cis* and *trans* isomers).	22	do	Blue	Yellow	1.34
	23				1.46
p-Hydroxybenzoic acid	22.1		Deep blue	White	1.42
3, 4-dihydroxybenzoic acid	24.9		Blue	Light yellow	1.60

[1] Mobility relative to hydroxymethylfurfural and picric acid.

	R_1	R_2	R_3
Vanillic acid	OCH_3	H	OH
Vanillin	OCH_3	H	H
3, 4-Dihydroxybenzoic acid	OH	H	OH
3, 4-Dihydroxybenzaldehyde	OH	H	H
p-Hydroxybenzoic acid	H	H	OH
p-Hydroxybenzaldehyde	H	H	H
Syringic acid	OCH_3	OCH_3	OH

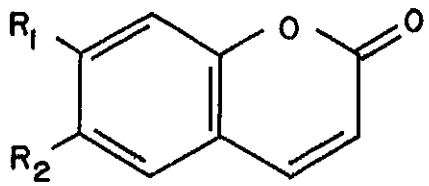
Up to this point none of the compounds is colored, and so can only be considered pigments in the broader sense. However, these compounds do have flavor and odor, and so can have deleterious effects on products made from the sugar. Some users of sugar specify low sugar color not because they need a white sugar, but because they need low flavor and odor. There is no convenient measure of these properties, but color can be measured.

Six of the compounds are cinnamic acid derivatives, their corresponding alcohols, or esters.



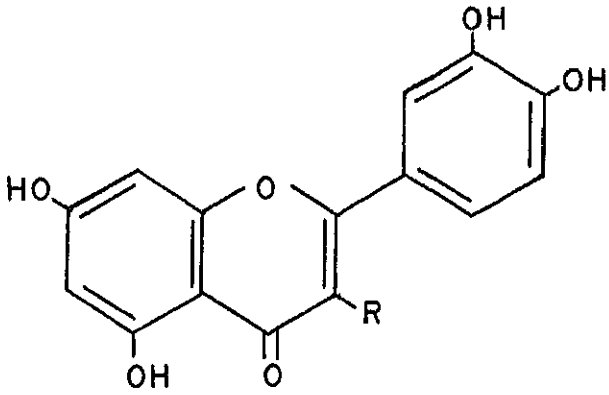
	R_1	R_2	R_3
p-Coumaric acid	OH	H	H
Caffeic acid	OH	OH	H
Ferulic acid	OH	OCH_3	H
Sinapic acid	OH	OCH_3	OCH_3
Chlorogenic acid	OH	OH	H quinic acid ester
Coniferin	O-glucose	OCH_3	H alcohol

Three of the compounds are coumarin derivatives, but coumarin can be considered the product of ring closure of *o*-hydroxycinnamic acid.



	R_1	R_2
Coumarin	H	H
Umbelliferone	OH	H
Esculin	OH	O-glucose

Three of the compounds are flavonols.



	R
Kaempferol	H
Quercetin	OH
Rutin	O-rutinose (=rhamnose glucose)

These last three classes of compounds are all colored, generally yellow, and more strongly colored at higher pH. The flavones are usually more deeply colored than cinnamic acid derivatives. It might be of interest that in some plants an inverse relationship has been found between flavones and cinnamic acid derivatives: the more of one the less of the other in mutations of the plant (*9*).

CONCLUSIONS

The colorants that have been identified are undoubtedly only a small fraction of all the compounds present. Many of the others will surely be other derivatives in these same classes, but it will be interesting to see what other classes of plant pigments are discovered.

There are two additional classes of colorants, not plant pigments, which might account for a large fraction of the sugar colorant. These are reaction products of amines with reducing sugars (melanoidins) and decomposition products of sucrose (caramels). The evaluation of their relative importance in sugar refining depends upon their identification and the development of a method of measuring them so that they can be followed through the commercial processes. But, the first step is to identify the colorants.

REFERENCES

(1) Bjerager, E., and Bruniche-Olsen, H. 1968. Preliminary investigations on extraction of sugarcane at varying pH values, temperature, and retention times. Proc. Int. Soc. Sugar Cane Technol. 1968: 151–164.

(2) DeLaunoy, O. 1962. Chromatographic study of the phenol derivatives in a beet molasses. Sucre Belge 82: 241–250. (Sugar Ind. Abstr. 63: 505.)
(3) De Roubaix, J., and Lacar, O. 1957. The inhibitory substances contained in sugar beet glomerules. Sucre Belge 5: 185.
(4) Farber, L., and Carpenter, F. G. 1971. Chlorogenic and caffeic acids identified as colorants in cane sugar. Int. Sug. J. 73: 99.
(5) ——— and Carpenter, F. G. 1971. Identification of sugar colorants. Proc. 1970 Tech. Sess. Cane Sugar Refining Res., pp. 145–156.
(6) ——— and Carpenter, F. G. 1972. Indentification of cane pigments that persist into refined sugar. Proc. Int. Soc. Sugar Cane Technol. 1971: 1589–1600.
(7) ——— Carpenter, F. G., and McDonald, E. J. 1971. Separation of colorants from cane sugar. Int. Sugar J. 73: 170–173.
(8) ——— McDonald, E. J., and Carpenter, F. G. 1969. Separation of colorants from cane sugar. Proc. 1968 Tech. Sess. Cane Sugar Refining Res., pp. 85–104.
(9) Geissman, T. A., and Harborne, J. B. 1955. The chemistry of flower pigmentation in *Antirrhinum majus*. IV. The albino (—mn —nn) form. Arch. Biochem. Biophys. 55: 447–454.
(10) Gillett, T. R. 1953. Color and colored nonsugars. *In* Honig, P. (ed.), Principles of Sugar Technology, Vol. I, pp. 214–285. Elsevier, Amsterdam.
(11) Gross, D. 1961. An apparatus for high-voltage paper electrophoresis. J. Chromatogr. 5: 194–205.
(12) ———. 1957. Electrophoretic studies on colored compounds. Int. Sugar J. 59: 339–342.
(13) Hashizume, T., Yamagami, T., and Sasaki, Y. 1967. Constituents of cane molasses. 2. Separation and identification of the phenolic compounds. Agric. Biol. Chem. (Tokyo) 31: 324–329. (Sugar Ind. Abstr. 67: 1016.)
(14) Ito, H., Kagabu, K., and Kamoda, M. 1965. [Studies on the odour of molasses. 1. Volatile organic acids and phenols in refinery molasses]. Proc. Res. Soc. Japan Sugar Refineries' Technol., 15: 56–61. [In Japanese] Sugar Ind. Abstr. 65: 426.
(15) McLaren, A. 1954. A tentative method for the quantitative analysis of colored compounds in cane sugar products. (Abstracts) ACS 126, 21D; Sugar Ind. Abstr. 54: 725.
(16) Obata, Y., Senba, Y., and Koshika, M. 1963. Detection of phenolic compounds by chromatography in beet sugar molasses. Agric. Biol. Chem. (Tokyo) 27: 340–341. (Sugar Ind. Abstr. 63: 962.)
(17) Parthasarathi, K., and Vijayasaradhy, M. 1958. On the phenolic content of sugarcane and saccharum spontaneum juices. Curr. Sci. 27: 218–219. (Sugar Ind. Abstr. 58: 715.)
(18) Pratt, D. D., and Robinson, R. 1922. A synthesis of pyrylium salts of anthocyanidin type. J. Chem. Soc. 121: 1577–1585.
(19) Roberts, E. J., and Martin, L. F. 1960. Progress in determining organic nonsugars of sugar cane juice that affect sugar refining. Proc. Tech. Sess. Bone Char 1959. p. 67.
(20) Seshadri, T. R. 1960. Coloring matter of the sugar cane and sugar beet. Sharkara 3: 83–89. (Sugar Ind. Abstr. 61: 178.)
(21) Smith, P., and Hall, P. M. 1971. Sugarcane anthocyanins as colour precursors and phytoalexins. Proc. Int. Soc. Sugar Cane Technol. 1971: 1139–1146.
(22) Stevens, R. 1959. Phenolic constituents of sugar cane. Int. Sugar J. 61: 199. (Sugar Ind. Abstr. 59: 954.)
(23) Tu, C. C., and Onna, K. 1960. The nonsucrose constituents of Hawaiian raw cane sugar crystals. Proc. Int. Soc. Sugar Cane Technol. 1959: 291–304. (Sugar Ind. Abstr. 60: 1158.)
(24) Underwood, J. C., and Filipic, V. J. 1964. Source of aromatic compounds in maple sirup flavor. J. Food Sci. 29: 814–818.
(25) Van Sumere, C. F. 1960. Germination inhibitors in plant material. *In* Pridham, J. B. (ed.), Phenolics in Plants in Health and Disease, pp. 25–34. Pergamon Press, New York.
(26) Wickberg, B. 1956. Investigation of melamine-forming substances in *Beta vulgaris*. Socker Handl. I., 12: 169–175. (Sugar Ind. Abstr. 57: 372.)
(27) Willstätter, R., and Everest, A. E. 1913. Untersuchungen über die Anthocyane. 1. Über den Farbstoff der Kornblume. Annalen 401: 189–232.

DISCUSSION

P. H. PETRI (Godchaux-Henderson Sugar): Were all these colorants found in refined sugar?

F. G. CARPENTER: No, about half of them were found in refined sugar. Table 3 shows where the various colorants occur. Other colorants disappeared during refining—in affination, clarification, or on bone char. Some are removed from cane juice before raw sugar is crystallized out, but not all of them are easy to remove; as the old assumption had it, that plant pigments were no problem.

W. L. REED (Revere): Dr. McDonald, who used to be with the Project, isolated two components called C–1 and C–2. Are these among your constituents?

F. G. CARPENTER: Yes. Dr. McDonald made a column chromatography separation and got bright yellow bands which she called C-1 and C-2. They were the first two components off the column, and may be related to Dr. Smith's yellow spots (mentioned in the first paper) which he got out later from his columns. C–1 and C–2 are also in the lower molecular weight range. She was able to pin those down to some extent. She identified C–2 as a flavone of flavonol with four OH's, and some other group, but she was not sure

where the R-group was. The flavone with no R is apigenin; and Dr. McDonald's compound, which became spot No. 10 on our spot numbering system, was found to be rutin, which has in the R position a rhamnose and a glucose.

N. H. SMITH (California and Hawaiian): I have estimated that about half of the color of raw sugar is naturally occurring pH-sensitive colorants. I have tried comparing the spectra of sugars with the spectra of known pigments and they never quite match up. In the natural state, there are probably sugars attached to various points that can give them different spectral properties. I have a question, one that I am frequently asked: Given a pile of sugar, how soon can we say that it contains $X\%$ of compound G or whatever? We cannot actually analyze for these things as yet.

F. G. CARPENTER: We cannot yet analyze for them; however, the first step is to identify them. You can find cases in the literature, and you heard two more papers this morning, where attempts were made to analyze for unknown substances. The analysis is really for groups of materials with similar properties, and the results are never quite satisfactory. So the first step is to identify the materials and then to develop methods for analyzing for them. In a paper this afternoon and one tomorrow, we will go into the analytical methods. Many of these pigments are fluorescent, so one obvious analytical method is to use fluorescence measurements. The other tried-and-true method which everybody goes to these days is gas chromatography, which is very sensitive to small amounts of material. We will have another paper on that. We still are not at the point where we can take, as you say, a sample of sugar off the ship and analyze it for all of these different compounds, but we are approaching that point. On the other hand, we may not want to analyze it for all of these compounds, because there are too many. Take, for example, sugar color. Color is three dimensional, but nobody uses a three-number sugar color scale. Everybody uses a one-dimensional sugar color scale, because three numbers are too complex in practice. Now, if three numbers are too complex, the analysis for many different compounds that may run even to 100 will certainly be too much to digest. On the other hand, we do have computers. We will probably have to analyze for groups of materials (known groups, that is), such as benzoic acids, or cinnamic acids, or maybe plant pigments as a group, as compared to something else. I believe that the day will come when we will be able to take a sample of sugar off the ship and run it through an analysis and be able to say that when this sugar gets in to the refinery we will have to adjust the controls in a certain way, a little longer cycle some place, slow down the flow rate here, increase it there, burn more char, use less phosphoric acid, and so on. It will be a happy day.

W. W. BINKLEY (New York Sugar Trade Laboratory): Taste panels tell us that sugar from different refineries have different taste properties, such as a chemical taste or metallic taste. These constituents that you refer to today, have they been taste tested?

F. G. CARPENTER: We have not actually studied them for taste, but you recognized some of those names as known flavors, vanillin for instance. It is probably one of the molasses flavors or odors or both. And speaking of molasses, I try to avoid getting fooled by molasses. Molasses is what you throw away. That is what we do not need to worry about anymore. What we have to worry about are the things that are not in the molasses but remain in the sugar. Just because there is a large amount of something in molasses does not mean that something is a big item. There might be a little bit in the sugar, but the biggest item in molasses is not necessarily what we have to refine out of the sugar.

W. W. BINKLEY: I would like at this time to philosophize a little, specifically on the subject of melanoidins. After two decades of work and over 30 published papers on melanoidins, I have decided I do not know what they are. I have come to the conclusion that the solution to this phase of the color problem is to find a chemical way of getting rid of melanoidins. For characterization, I can tell you how many hydroxyl groups are in the particular melanoidin, and how many phenolic groups, and can even give you a rough molecular weight; but, I feel that the effort in the future should be directed, perhaps it has already begun, at finding methods to simply reduce these polymers to smaller substances which will not engage in further melanoidin reactions.

F. G. CARPENTER: I think we have heard some words of wisdom from the man who knows more about melanoidins than anybody in the world, so those of us who are in research should bend our efforts in that way.

CHARACTERISTICS OF RAW SUGAR FROM SWEET SORGHUM

By B. A. Smith, R. C. Smith, R. V. Romo, R. A. de la Cruz, and B. J. Lime[1]

(Presented by B. A. Smith)

ABSTRACT

Results of recent research suggest that the commercial production of raw sugar from the juices of sweet sorghums will prove attractive to the sugar industry. Pilot-plant studies of processes similar to those employed with sugarcane juices and sirups indicate that although there are differences in the composition of juices from sweet sorghum and sugarcane, the raw sugars crystallized from them are similar except for the levels of the various mineral constituents. These differences in mineral contents, the distribution of the mineral constituents in the sugars, and comparisons with the levels found in the sirups and molasses are discussed.

INTRODUCTION

The possibility of recovering crystalline sugar from the juices of the sweet sorghums [*Sorghum bicolor* (L.) Moench] has interested U.S. farmers and sugar producers since the seeds of these plants were introduced to this country from Africa and China about the middle of the 19th century. The adaptability of these plants to the soils and climates of extensive areas of the United States, their rapid growth, and their modest water requirements attract the farmer. However, the lack of suitable disease-resistant, high-yielding varieties of sorghum and the failure of existing sugarcane and sugar beet processes to eliminate the unusual quantities of starch and aconitic acid in the sweet sorghum juices have severely handicapped all commercial efforts to produce crystalline sugar from the crop (*3, 11, 22*).[2]

The USDA's Agricultural Research Service, through its Food Crops Utilization Research Laboratory at Weslaco, Tex., undertook research on the sweet sorghums in 1964 in cooperation with Texas A&M University's Research and Extension Center at Weslaco, and with the ARS Sugar Crops Field Station at Meridian, Miss. The objectives of this joint research effort are to evaluate new breeding lines of sweet sorghum with potentials for commercial sugar production and to investigate means of reducing the starch and aconitic acid contents of the raw juices to levels that do not interfere with the crystallization of sucrose. The release in 1965 (*2*) of a new disease-resistant, high-sucrose sorghum, 'Rio', gave impetus to this research effort.

In 1969 and 1970 we described the results of laboratory experiments on the elimination of starch from the raw juices by procedures which were adaptable to sugarcane factory operations and which did not necessitate the use of amylolytic enzymes (*14, 17*). Over 90% of the starch was eliminated during raw juice clarification by the following procedure: (1) maintaining juice density below 16 Brix, (2) liming to 7.7–7.9 pH range, (3) regulating temperature at the 50°–55° C range and (4) adding approximately 5 parts per million (p/m) of flocculating agent. An additional 8% of the starch content was removed by clarification of the 30- to 40-Brix semisirups by the following procedure: (1) liming to pH of 7.1–7.3 if necessary, (2) heating to 60°–70° C, and (3) adding 3–5 p/m of flocculating agent and settling of insoluble material (*15*). Emil K. Ventre et al., in a publication (*20*) and a patent (*19*), described means of crystallizing the insoluble dicalcium magnesium salt of aconitic acid and separating it from sweet sorghum sirups, a process which has promise of practical commercial application.

In 1970, pilot-plant batch tests of these proce-

[1] Chemists, Food Crops Utilization Research Laboratory, Southern Region, Agricultural Research Service, U.S. Department of Agriculture, Weslaco, Tex. 78596.

[2] Italic numbers in parentheses refer to items under "References" at the end of this paper.

dures for removing starch and aconitic acid were undertaken at Weslaco. Clarified juices were processed to sirups, and A, B, and C sugars were crystallized satisfactorily from the product sirups and molasses. Examination of the various juice- and sirup-processing fractions and of the sugars and molasses substantiated the satisfactory laboratory eliminations of both starch and aconitic acid (*16*). Results of the sugar-boiling work in 1970 were not altogether satisfactory because the investigators' lack of experience with vacuum sugar pans and because of the high mineral content of the raw juices, sirups, sugars, and molasses. The raw juices extracted from sweet sorghums grown on the high-potash soils of the Weslaco area were found to contain from 5% to 8% carbonate ash on juice solids, much higher than the values of 2% to 5% reported elsewhere (*18*, *21*). Although, with repeated cropping, the sweet sorghum plants grown on these soils might be expected to produce juices of lower mineral content, as has been noted with sugarcanes grown in new sugar-producing areas, the possibility of reducing ash levels during processing seemed worth exploring. Results of the 1971 pilot-plant tests are reported here, with particular emphasis on those procedures which would affect the quality of the raw sugars.

MATERIALS AND METHODS

Juice and Sirup Processing Studies

Juices and sirups were clarified by allowing the insoluble sediments to settle out; the clear liquids were siphoned off. Operating conditions such as juice density, pH, temperature, and flocculant usage, mentioned in the "Introduction," are described in detail in an earlier publication (*16*). Juices and sirups were concentrated in the second effect of a double-effect evaporator. All processing steps preceding the treatment of the 60- to 65-Brix sirups for crystallization of the insoluble aconitic acid salt were essentially the same as those described for 1970 tests.

The insoluble dicalcium magnesium aconitate was crystallized by the process described by E. K. Ventre (*19*, *20*), except that a small quantity of $MgCl_2$ was added in case the sirups were deficient in Mg. Briefly, the conditions Ventre recommended for elimination of approximately 80% of the aconitic acid were (1) adjusting the concentrated sirup pH to 8.0–8.3 with lime, (2) heating to boiling, (3) adding a $CaCl_2$ solution to provide 60% of the stoichiometric amount of Ca needed for reaction with the aconitic acid present, and (4) settling the insoluble salt. Under these conditions crystallization and precipitation of the insoluble aconitate were satisfactory, but the carbonate ash of the sirup was increased.

Later examination showed that the untreated heavy sirups processed in 1970 contained more than half of the Ca needed for formation of the insoluble aconitate and suggested that the added 60% of the stoichiometric requirement of Ca as $CaCl_2$ was probably more than was needed. Hence for the 1971 tests, assuming that the untreated sirups contained essentially the same level of Ca as that found in 1970 materials, the $CaCl_2$ additions were reduced to provide 10% to 25% of the stoichiometric requirement of Ca. Under these conditions the crystallization of the aconitate was satisfactory, and since both the Ca and Mg contents of the sirup had been depleted, the ash of the sirup was reduced by 0.25 percentage points, sirup purity was improved, and suspended matter was significantly decreased.

The raw juices available for the 1971 pilot-plant tests were of average quality, compared to area juices since 1964, and were probably representative of juices from well-managed field materials grown on the Weslaco soils. The true purities of the raw juices averaged 78.74% and increased to 83.42% on the finished sirups. Carbonate ash on raw juice solids averaged 6.68%, increasing to 7.08% on clarification, and decreasing to 6.43% on finished sirup. Starch at 2.12% on raw juice solids decreased to 0.014% on finished sirup solids. Aconitic acid on juice solids averaged 1.96% and decreased to 0.55% on finished sirup solids.

The finished sirups were used in boiling nine A strikes, and A and B molasses provided materials for boiling three B and three C strikes in the pilot-plant sugar pans. All massecuites, sirups, sugars, and molasses were weighed and analyzed. True and apparent purities of sirups and molasses, as well as the apparent purities of massecuites were determined. Pol values on the sugars were adjusted to the ovendry weight of sugar. Aconitic acid was measured by the method by Poe and Barrentine (*12*), and starch values were determined by the methods of Dubois et al.

(4). Carbonate ash was the weight of residual material after incineration at 560° C, and can be converted to sulfate ash satisfactorily by using the factor 1.28 × carbonate ash. Chlorides and sulfates were determined by titrimetric procedures. The carbonate ash samples were submitted to a commercial laboratory for measurement of K, Ca, Mg, Na, Fe, SiO_2, and P_2O_5. All but two were measured by atomic absorption spectrophotometry; SiO_2 and P_2O_5 were determined by gravimetric and colorimetric procedures, respectively. As a suitable basis for comparison of the data from these tests, the dry solid content of all samples was determined by vacuum oven-drying on sand at 59° C.

Sugar Boiling Tests

Two vaccum sugar pans were used for boiling all sugars. The smaller was a modern calandria pan, with a capacity for approximately 2½ ft^3 of massecuite, patterned after the experimental unit developed by Dr. Arthur G. Keller of Louisiana State University, and had a 90-r/min stirring device. This pan was used in boiling all A and B strikes. The larger unit, with a capacity of approximately 4 ft^3 of massecuite, was an old coil pan with four steam coils and no means of mechanical stirring. Large C strikes were boiled in this unit.

An insulated 3-ft^3 crystallizer with a 12-inch conveyor trough and paddles moving at 3 r/min was used for curing C strikes before centrifugation. A and B massecuites were transferred to an elevated tank for gravity discharge into the centrifuge. C massecuites from the crystallizer were discharged in batches suitable for one centrifuge load, then warmed rapidly to 105°–110° F before the centrifuge was charged. The centrifuge was an 18-inch-diameter, perforated-basket unit fitted with a slotted sugar screen and was operated at 1,200 r/min for purging and at 20 r/min for discharging sugar through removable bottom plates.

A strikes were boiled with C sugar and a sirup footing, and the crystals were grown in sirup. B sugars were produced from a footing provided by a grain strike and A molasses, and were grown with A molasses. C strikes were produced with a footing from a grain strike and B molasses, and were grown on B molasses. The grain strikes were specially boiled from sirups and seeded with powdered refined sugar suspended in isopropyl alcohol. A strikes required 5 to 6 hours for completion, B strikes about 4 hours, and C strikes about 6 hours. Boiling was conducted at approximately 150° F under a vacuum of 26 inches Hg.

A and B sugars were sprayed in the centrifuge with a small amount of water mist to help purge molasses from the sugar crystals. A spray of steam was used for purging C sugars. Washing of the crystals in the centrifuge was kept to a minimum to avoid redissolving the crystals. Raw sugars were dried in a forced-air convection oven at 80° to 90° C before weighing, screening, and bagging.

The sugar crystals produced in these experiments were small size since production of the larger crystal sizes, preferred by most cane sugar factories, would impose serious problems for intermittent pilot-plant operation. Too, the extent of washing or purging of molasses films from the crystals was doubtless quite variable in spite of efforts to make these operations uniform. Hence no claim can be made that these sugars could be considered as representative of commercial raw sweet-sorghum sugars, although the data suggest that they should approximate the composition of commercial sweet-sorghum raws, with the possible difference that the raw sugars produced commercially would be expected to contain less mineral matter.

RESULTS AND DISCUSSION

The data from the sugar-boiling experiments in table 1 show the performances obtained in the small sugar pans. Purity drops in A and C strikes were reasonably uniform, but with B strikes the smaller drop appears to reflect a difficulty encountered in handling these materials. Percent recovery of massecuite sucrose as raw sugar emphasizes the fact that less sugar was crystallized from the B massecuites. Two experiments (not reported in table 1), in which A and B massecuites were cured in the crystallizer before centrifugation, indicated that final molasses could be exhausted to the same degree by such a two-

TABLE 1.—*Sugar-boiling performance*

	A strikes	B strikes	C strikes
Apparent purity, massecuites, % ..	79.7	67.7	61.8
Apparent purity, molasses, %	64.2	58.3	45.1
Purity drop, percentage-points ...	15.5	9.4	16.7
Crystallization, %	57.0	35.7	57.4

strike system as with the more tedious three-strike schedule followed during these experiments. The 45.1% average apparent purity of C molasses reflects the limiting effect of high mineral content on the exhaustibility of these materials.

The composition of the molasses and sugars produced from these sugar-boiling experiments is expressed as percentages on dry solids (table 2). True purities of the molasses are reported here, and the Pol values for the sugars are adjusted to the dry basis. Although values for reducing substances were not determined on the sugars, the sum of Pol and sulfated ash values suggests levels of approximately 0.4%, 1.0%, and 1.3% for sugars A, B, and C, respectively. These values correspond reasonably well to contents measured chemically on 1972 raws. Starch contents reported here for A and B sugars, which constitute the materials sold as commercial raws, were much lower than the 147-p/m average found in affined South African raws from nine factories in 1969 when no specific starch removal procedures were employed (*10*), and also significantly lower than starch values noted by Charles (*1*) on washed raw sugars of various origins received at the Crockett Refinery. Although colors were not measured routinely, the color of a representative lot of blended A, B, and C sugars was reported as satisfactory, measuring 139 at 560 nm and 592 at 420 nm according to American Sugar Refining Company's Contract No. 10 for affined sugar color. Filterability of this same lot of affined blended A, B, and C sugars by the same Contract No. 10 specifications was reported to be satisfactory at 135.[3]

Determination of the alcohol precipitable gum content of two of the sweet sorghum raws by the Roberts-Friloux method (*13*) indicated levels of 780 p/m on an A and 1,880 p/m on a C sugar, whereas two Louisiana commercial raws contained 1,420 and 3,580 p/m, a Louisiana C sugar contained 5,630 p/m and two offshore raws contained 1,250 and 1,850 p/m.[4] In a report on raw cane sugars imported into South Africa during 1965 from five sources, Jennings (*9*) found gum contents ranging from 1,300 to 2,300 p/m. The quantities of gums found in the raw sorghum sugars are compatible with the amounts of alcohol insolubles found in the sweet sorghum juices, which ranged from 0.19% to 0.30% on solids, and did not increase significantly in the sirups. Final molasses gum contents averaged 0.52% on solids, much less than is usually encountered in blackstrap produced from sugarcane.

One sweet sorghum A and one C sugar were examined by Mrs. Mary A. Godshall of the Cane Sugar Refining Research Project in New Orleans. Gas-liquid chromatography of sorghum C and a Louisiana raw showed no unusual differences, although there seemed to be fewer constituents and in smaller quantities in the sorghum C sugar, and even a little less aconitic acid than in the cane sugar. By electrophoresis definite differences were detected in the sweet sorghum A sugar and the Louisiana sugarcane raw. A somewhat different spectrum of colorants was detected in the two sugars, but the total amount of colorants was about the same in each sugar.

The distribution of individual mineral constituents in the A, B, and C sugars is shown in table 3, expressed as percentages on dry sugar. KCl is the principal salt present in all of the sugars, with aconitates second. The rather insignificant quantities of CaO and MgO reflect the effectiveness of the aconitic acid removal in depletion of these constituents. Before this aconitic acid removal the sirups contained CaO and MgO at levels of 0.44% and 0.19%, respectively; after removal the levels were 0.08% and 0.09%, re-

[3] Private communication, A. M. Bartolo and L. Anhaiser, Imperial Sugar Co., Sugar Land, Tex.

[4] Private communication, J. E. Irvine, USDA's Sugar Crops Field Station, Houma, La.

TABLE 2.—*Composition of molasses and sugars*[1]

Component	A		B		C	
	Molasses	Sugar	Molasses	Sugar	Molasses	Sugar
True purity	70.76	98.94	65.59	97.05	52.83	89.45
Reducing substances	9.18	.....	10.50	.....	15.93	.....
Starch	.021	.003	.028	.006	.041	.009
Carbonate ash	12.69	.38	14.99	1.43	16.95	7.18

[1] All values reported as percentages on dry solids.

TABLE 3.—*Mineral composition of raw sugars, percent dry sugar*

Component	A sugars	B sugars	C sugars
K_2O	0.179	0.777	3.825
CaO	.009	.015	.031
MgO	.008	.020	.039
Na_2O	.005	.009	.021
Fe_2O_3	.005	.012	.010
Cl	.151	.583	3.697
Aconitate	.043	.070	.200
SO_4	.003	.047	.055
SiO_2	.011	.019	.075
P_2O_5	.003	.006	.0009

spectively. All materials processed in the evaporator, sugar pans, or crystallizer frequently contained more Fe_2O_3 after processing than before, since these units were all subject to extensive scaling of iron rust as a result of intermittent usage. The quantities of all cations and anions found in these sugars increased rapidly from A to C strikes, largely in response to the rapid increase in concentration of these constituents in the A and B molasses from which the sugars were crystallized. A secondary effect, which accentuated the large increases in mineral content of the sugars from A to C strikes, was the increasing difficulty in purging molasses from the B and C sugar crystals with limited washing. The B and C sugars, as discharged from the centrifuge, contained significantly greater quantities of high-ash molasses. These sugar crystals were small, and double purging in the manner often employed to reduce nonsucrose levels in low Pol raw sugars was not a regular practice; however, in two experiments where C sugars of 7% ash were double-purged, ash levels were reduced approximately 50%.

Table 4 shows the distribution of mineral constituents between the sweet sorghum sirups and their product A sugars, and, for comparison, similar data from cane sirups and their product A sugars, both raw and affined, as reported by Fort in 1938 (*5*). Differences in the conditions under which the cane and the sweet sorghum sugars were crystallized and in subsequent handling make such comparisons difficult.

Although true purities of the cane and sweet sorghum sirups were essentially the same at 83.29% and 83.42%, respectively, and the percentages of sirup sucrose recovered in the raws were very similar at 56.2% and 57%, respectively, comparison of the sugars produced from these sirups is handicapped by the procedures employed in separating the molasses from the crystals in the centrifuge, which in turn affected the ash composition of the raw sugars. The sweet sorghum sugars were washed with a small amount of water during centrifugation to purge molasses from the crystals whereas the cane raws were not washed at all.

TABLE 4.—*Distribution of mineral constituents between sirups and product sugars*[1]

Constituent	Sugarcane[2]			Sweet sorghum	
	Sirup	Raw sugar	Affined sugar	Sirup	Raw sugar
Carbonate ash	3.724	0.709	0.0897	6.43	0.380
K_2O	1.851	.333	.0402	3.38	.179
CaO	.308	.057	.0137	.08	.009
MgO	.182	.030	.0022	.09	.008
Na_2O	.....	.....	.....	.07	.005
Fe_2O_3	.0011	.0005	.00015	.01	.005
P_2O_5	.....	.....	.....	.02	.003
SiO_2	.030	.028	.0050	.09	.011
Aconitic acid	.....	.....	.....	.55	.041
SO_4	.811	.144	.0185	.15	.003
Ci	.....	.....	.....	2.20	.151

[1] Values reported as percent dry solids.
[2] Sugarcane data reported by Fort (*5*).

The third column in table 4, showing mineral constituents of the affined cane raw, indicates the quality of the sugar to be melted by the refiner. Since affination is more efficient than washing in the centrifuge as a procedure for removing the high-mineral molasses film from the crystals, the sorghum sugar may be considered as intermediate in quality between an unwashed and an affined sweet sorghum raw sugar. This assumption appears warranted in view of the percentages of sirup ash which are found in the different sugars. Nineteen percent of the cane sirup ash is found in the unwashed raw and 2.4% in the affined raw, whereas 5.9% of the sorghum sirup ash is found in the lightly washed raw sugar.

The major ash constituent in both cane and sweet sorghum materials is K_2O, but the distribution pattern of all mineral constituents in the product sugars is largely dependent on the quantity found in the massecuite and the efficiency with which the high-ash molasses film is removed from the sugar crystal.

Honig (*7, 8*) found that K_2O, MgO, Na_2O, and Cl were removed to a greater degree by washing or affination of the raw sugar than CaO, Fe_2O_3,

SiO_2, P_2O_5, SO_4, and possibly aconitates, since the latter group was incorporated throughout the sugar crystal, whereas the former components were present principally in the film of molasses surrounding the crystal. Comparisons of the percentage retention of cane sirup constituents in the affined sugar tend to substantiate these findings, as 2% or less of the sirup K_2O and MgO contents, in contrast to 2% to 16% for SO_4, CaO, and SiO_2, were found in the affined sugar. Consideration of the sweet sorghum mineral constituents reveals that 5% to 9% of the K_2O, Cl, Na_2O, and MgO in the sirup, and 8% to 15% of the aconitate, CaO, SiO_2, and P_2O_5 remained in the washed sugars. The SO_4 remaining in these sugars was unusual at a retention level of 2% of the sirup content. The most striking differences between the two sugars compared in table 4 are the large quantities of K_2O and Cl, and the very small quantities of CaO, MgO, and SO_4 found in the sweet sorghum sugars, so that a significant decrease in total ash could be anticipated after affination.

Aconitic acid is the principal organic anion in Louisiana sirups, and the quantity present in these cane sirups doubtless exceeded the amount found in the sweet sorghum sirups. Because more CaO and MgO are present in the cane sirups, and because of the insolubility of dicalcium magnesium aconitate in hot massecuities, the quantity of aconitate retained in the raw cane sugar would be expected to be greater than in the sweet sorghum sugar. P_2O_5 and SiO_2 contents in the sweet sorghum sirups and sugars are in the ranges reported for South African sirups (*6*), whereas Fe_2O_3 contents are high because of the scaling effect noted previously.

CONCLUSIONS

Commercial raw sugars crystallized from sweet sorghum sirups and molasses should not differ notably in composition from the raw cane sugars which are being successfully refined today. Because of the clarity of the sirups from which they were crystallized, sugar melts from sweet sorghum should contain less suspended matter than those from cane raws. Proper control of raw juice and sirup processing has been found to reduce sirup starch contents to the level of approximately 0.01% to 0.02% on solids, and the product raw sugars at the level of 0.003% to 0.004% starch contain less of this objectionable constituent than most commercial raw cane sugars today. Aconitic acid eliminations can be controlled to provide raw sugar contents of 0.04% to 0.06%. The high content of carbonate ash in the raw juices, sirups, and sugars reported here reflects the high content of potash in local soils, and has not been reported in juices from other areas. Careful management of sugar boiling and centrifugation should permit reduction of the mineral content of the raw sugars to levels consistent with good refining characteristics. Fortunately, the high ash is due largely to KCl, which is not preferentially incorporated throughout the sucrose crystal. SO_4 and CaO, both objectionable in sugar refining, are found at very low levels in the sweet sorghum sugars. Insufficient data are available on the gum content, the identity and quantity of colorants present, and the filterability of the sweet sorghum raws, but there are indications that these sugars should compare favorably with sugarcane raws.

REFERENCES

(1) Charles, D. F. 1969. Quick starch method of analysis on raw sugars. Proc. 1968 Tech. Sess. Cane Sugar Refining Res., pp. 75–84.

(2) Coleman, O. H., Cowley, W. R., Broadhead, D. M., and others. 1965. Rio, a new disease resistant variety of sweet sorghum for Southern United States. Agric. Res. Serv. [Rep.] 34–72, 83 pp.

(3) Cotton, R. H., Norman, L. W., Rorabaugh, G., and Haney, H. F. 1951. Semipilot production of sucrose from sorghum. Ind. Eng. Chem. 43: 628–635.

(4) Dubois, M., Gilles, K. A., Hamilton, J. K., and others. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350–356.

(5) Fort, C. A. 1938. The influence of syrup composition on sugar quality. Proc. 6th Congr. Int. Soc. Sugar Cane Technol., pp. 1005–1018.

(6) Graham, E. W., Wright, B. F., and Alexander, J. B. 1959. The composition of South African syrups. Proc. S. Afr. Sugar Technol. Assoc. 33: 65–66.

(7) Honig, P. 1953. Inorganic nonsugars. *In* Honig, P. (ed.), Principles of Sugar Technology, Vol. I, pp. 291–360. Elsevier, New York.

(8) ———. 1959. The significance of nonsugars for the manufacture of refined sugars. Proc. 6th Tech. Sess. Bone Char, pp. 101–116.

(9) Jennings, R. P., and Van Keppel, R. 1966. The quality of imported raw sugars. Proc. S. Afr. Sugar Technol. Assoc. 40: 196–198.

(10) Jullienne, L. M. S. A., Matic, M., and Teokarovic, M. 1970. The mutual clarification project. Progress Report No. 2. Proc. S. Afr. Sugar Technol. Assoc. 44: 71–80.

(11) Olcott, Henry S. 1857. *In* Sorgho and Imphee, the Chinese and African Sugar Canes. A. O. Moore, New York.

(12) Poe, W. R., and Barrentine, R. F. 1968. Colorimetric method for the determination of aconitic acid in sorgo. Agric. Food Chem. 16: 983–984.
(13) Roberts, E. J., and Friloux, J. J. 1965. Determination of the soluble polysaccharides in sugarcane products. Sugar y Azucar 60(11): 66–67
(14) Smith, B. A. 1969. Process for removing starch from sorghum juices. U.S. Patent No. 3,442,704.
(15) ———. 1969. Process for removing starch from sweet sorghum juices. U.S. patent No. 3,464,856.
(16) ———, Romo, R. V., Smith, R. C., and others. 1971. Preliminary pilot plant studies of the production of raw sugar from sorghum. Proc. 14th Congr. Int. Soc. Sugar Cane Technol., pp. 1637–1644.
(17) ———, Smith, R. C., Romo, R. V., and others. 1970. Removal of starch from sweet sorghum juices. Sugar J. 32(12): 25–30.
(18) Ventre, E. K. 1940. Preliminary report of the crystallization of sucrose from juices of sorgo plant. Sugar J. 3(7): 23–30.
(19) ———,Ambler, J. A., Byall, S., and Henry H. C. 1944. Process for the extraction of aconitic acid from plant juices. U.S. Patent No. 2,359,537.
(20) ———, Ambler, J. A., Henry, H. C., Byall, S., and Paine, H. S. 1946. Ind. Eng. Chem. 38: 201–204.
(21) Walton, C. F., Ventre, E. K., and Byall, S. 1940. Effects of low topping and diastatic malt extract on composition and quality of sorgo sirup. J. Agric. Res. 60: 427–432.
(22) Wiley, Harvey, W. 1888. Record of experiments with sorghum in 1888. U.S. Dep. Agric. Chem. Bull. No. 20.

DISCUSSION

P. H. PETRI (Godchaux-Henderson Sugar): How about the economics? Can sorghum sugar by produced and sold at a price competitive with cane sugar or beet sugar?

B. A. SMITH: From our own data we cannot be certain, but agronomists engaged in field studies on the sweet sorghums assure us that this crop can be produced considerably cheaper than sugarcane. For the greater part, these evaluations are based on the facts that planting is inexpensive, the crop matures in about 3½ months and so permits other use of the land, and water requirements are low. Engineering advisers tell us that the production of sugar from sweet sorghums should not be more costly than that from sugarcane.

A. B. RIZZUTO (Amstar): Would you care to comment about the use of bacterial amylase to solve some of your starch problems?

B. A. SMITH: The use of bacterial amylases to hydrolyze starch in the raw juices has not been considered because of the large quantities of starch found in these materials, and because sedimentation is both inexpensive and highly efficient. We did compare bacterial amylase and semisirup clarification as alternative processes for removing the small quantity of starch which escaped raw juice clarification during pilot-plant tests in 1969. Results of these tests indicated that, when properly employed, the amylases could effectively eliminate starch. However, the minute starch granules which escaped raw juice clarification were often difficult to rupture even at elevated temperatures, and the enzymes are not effective on unruptured granules. Other factors which mitigated against enzyme usage were the turbidities of the product sirups, which usually were two to three times the turbidities resulting from semisirup clarifications, and the fact that clarification by sedimentation is one of the most inexpensive of sugar house processes.

M. C. BENNETT (Tate & Lyle): Could you go into a little more detail about the preevaporation stage of this process. After extraction of the sorghum juice on a conventional mill tandem, does it go straight to evaporation or do you have clarification as with mill juice?

B. A. SMITH: It goes directly to clarification in a manner similar to cane juice clarification.

M. C. BENNETT: That is basically a calcium phosphate clarification?

B. A. SMITH: Correct. We have twice the weight of mud solids usually obtained in cane juice clarification and these muds effectively trap all but the very small starch granules.

M. C. BENNETT: Is the calcium chloride precipitation of the aconitate done after evaporation?

B. A. SMITH: That is correct. The calcium chloride is added to the 60-Brix sirup, and following separation of the aconitate, the sirup goes to the sugar pan.

F. G. CARPENTER (Agricultural Research Service): In order to put this all in perspective, would you delineate what steps in your process are in addition to the standard cane process. Also, probably a cane mill operated on pilot-plant scale, with everybody taking care of it, could make good sugar too. But in commercial operations, where you do not have the same care and precautions, do you expect more difficulties with the sorghum sugar?

B. A. SMITH: The raw juice clarification of sweet sorghum is very similar to that for sugarcane, but we use a higher pH and a much lower temperature. The clarified juice is evaporated to 30- to 40-Brix sirup. Now, the second clarification is not common to the cane sugar industry, but it is done essentially under the same conditions as the first, at a lower pH of 7.1 to 7.3 on the semisirup, and with the temperature somewhat higher, between 60° and 65° C, since by this time we no longer have any of the fragile large starch granules. The remaining starch granules are somewhat tougher. The third step that is different is the precipitation of the calcium aconitate from the 60- to 65-Brix sirups. Cane processing does not require this, although as I recall, Godchaux Sugars many years ago did recover aconitic acid from their sugarcane B molasses and thereby improved their recovery of C sugars. It is critical for sorghum sugar production to remove the large quantities of aconitic acid present in these materials. (If you get the aconitic acid out you also get a very high clarity.) So, there are two steps in this process which are somewhat different from raw cane sugar production. An additional point for consideration is that these sirups have an extremely low viscosity. We have not measured it, but 65-Brix sweet sorghum sirup has a viscosity about equal to 45-Brix cane sirup.

R. S. PATTERSON (California and Hawaiian): On your pilot-plant scale, you used temperatures of 50° to 55° C, and later 60° to 65° C. Do you not think on a large scale you might have problems with deterioration from bacteria at the relatively low temperatures used?

B. A. SMITH: In pilot-plant studies we have been obliged to discontinue operations following the 60° to 65° C semisirup clarification. These materials settle overnight in a covered insulated tank as a matter of convenience. The next morning the clear sirup is concentrated to 60 Brix, calcium chloride is added, and the sirup is heated to boiling and again allowed to settle overnight. We have not found any deterioration during these long periods of standing, nor has there been any significant increase of alcohol-insoluble gums during processing from raw juice to 60-Brix finished sirup. In commercial practice, continuous raw juice clarification will be more rapid than similar cane juice clarification, whereas the semisirup clarification is even more rapid than that of the raw juice, so that both operations would require little more time than raw cane juice clarification. The high temperatures needed for removing the aconitic acid from 60-Brix sirup have served to prevent deterioration in the sirups before sugar-boiling operations.

W. W. BLANKENBACH (Chapman Associates): What other sugars are found in the sorghum? Do you have raffinose and invert sugar as well as sucrose?

B. A. SMITH: We have made no effort to identify any sugars present in the sweet sorghums other than sucrose and group measurement of "reducing substances." In a sense we have accepted the research by Ventre et al.,[1] which identified sucrose, levulose, and dextrose only. With an invert sugar value of approximately 1% on solids in these juices, levulose decreases to very low levels and can disappear entirely. Apparently the sweet sorghum plant metabolizes dextrose at a higher level than levulose for production of tissue material and starch as well as stored sucrose. Hence at high sucrose levels, the levulose content gets very low. This produces an unusual Pol value under these conditions so that we prefer true purities over apparent values. When invert sugars amount to a more normal 3% to 5% on solids, the imbalance of dextrose: levulose does not appear to be so unusual.

M. C. BENNETT: To return to the question of low-temperature clarification: I wonder if you are familiar with a process that came into South Africa very briefly about the mid-1960's. This was developed by Rabé at Umzimkulu mill,[2] and was a cold mill juice clarification process designed specifically to operate below the temperature at which starch granules swell and gelatinize. The whole object of the exercise was to make sure the starch was still insoluble, in granules, so that it could be separated with the mud. You presumably operate your cold juice clarification of sorghum below starch gelatinization temperatures also. Rabé used a flotation process and that made it quite expensive, because it used polyacrylamides in very large quantities. The South Africans later found the enzyme solution to their problems.

[1] Ventre, E. K., Byall, S., and Catlett, J. L. 1948. Sucrose, dextrose, and levulose content of some domestic varieties of sorgo at different stages of maturity. J. Agr. Res. 76; 145–151.

[2] Rabé, A. E. 1964. Filtrate recirculation eliminated at Umzimkulu. 38th Proc. S. Afr. Sugar Technol. Assoc., pp. 110–113.

TRACE ELEMENTS IN SUGARS

By Philippe Pommez[1] and Margaret A. Clarke[2]

(Presented by Philippe Pommez)

ABSTRACT

Trace elements, particularly heavy metals, were analyzed for in a raw and a refined sugar by spark mass spectrometry, atomic absorption spectrophotometry, flame photometry, calorimetry, and polarography. The refining process used (carbonatation and bone char) lowered the levels of 20 heavy metals to far below the limits set by U.S. Government and other regulatory agencies. This information is timely in view of increasingly tight controls on levels of heavy metals in foods.

INTRODUCTION

The presence of trace elements in food products is of tremendous topical interest, particularly to the sugar industry, an increasingly frequent target of nutrition crusaders. An investigation into the nature and amounts of trace elements, particularly heavy metals, in sugar products is extremely important at this time.

Raw and refined sugars have been analyzed by various methods: spark mass spectrometry,[3] atomic absorption spectrophotometry,[4] flame photometry,[5] colorimetry,[5] and polarography.[5]

In this paper, these results are reported and compared with results from the literature (*6, 9, 12–18, 20*).[6] The toxic or nutritional nature of the metals, the regulations governing their use in food products, and their relation to levels of metals in common food products are all considered. All mineral nonsugars in raw and refined sugars were analyzed by spark mass spectrometry to determine effects of the refining process on trace elements. Some elements that are not classified as heavy metals were included in this survey.

NUTRITIONAL OR TOXIC METALS OF INTEREST

The elements that are included in our "heavy metal" classification (including some nontransition elements for the purpose of this study) are shown in figure 1. Those of particular interest because of their toxicity, nutritional value, or known presence in sugar products are crosshatched. Almost any one of these elements might be the next "scare word" in foods.

Table 1 lists the metals of interest that are essential for human nutrition (*1, 7*). These elements have all been found in sugar products. In general, to perform the functions listed here, these elements must operate as parts of metalloenzymes or as metal-activated enzymes. The fact that all of these essential trace elements have been reported to be found in refined sugar is a plus for the industry; however, since many of them are toxic if present in excess of the nutritional requirements, a quantitative estimate is needed.

Most heavy metals are toxic if present above certain levels. The difficulty of establishing levels of toxicity was stated by the FAO/WHO Expert Committee on Food Additives: ". . . for this purpose it would be necessary to take into account not merely the normal dietary variations and existing administrative limits in various countries, but also the following: the different toxic potentialities of the various elements; the epidemiological, environmental and experimental observations on variations in the occurrence

[1] Director of research, Redpath Sugars, Ltd., P. O. Box 490, Montreal, Canada.

[2] Administrator, Cane Sugar Refining Research Project, Inc., P. O. Box 19687, New Orleans, La. 70179.

[3] Laboratoire d'Analyses Physique, Paris, France.

[4] Southern Regional Research Center, New Orleans, La.

[5] Redpath Sugars, Ltd., Montreal, Canada.

[6] Italic numbers in parentheses refer to items under "References" at the end of this paper.

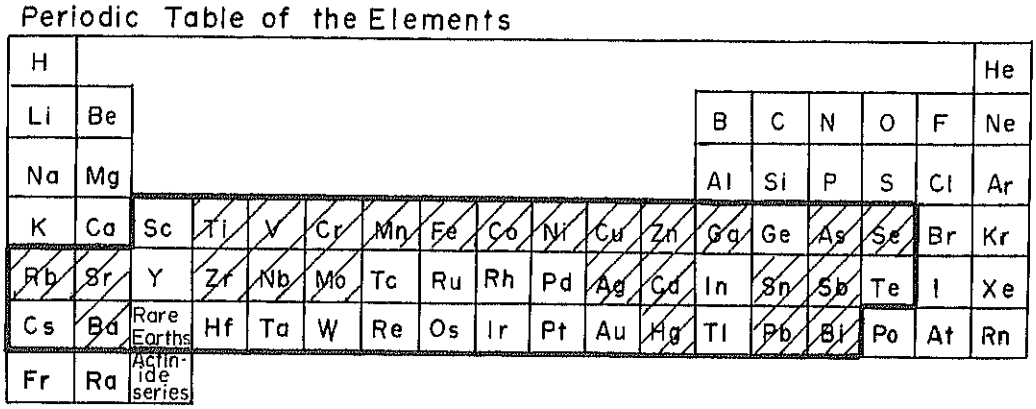


FIGURE 1.—Periodic table of the elements. The heavy metals as a class are enclosed in the bold line, and those considered here are crosshatched.

TABLE 1.—*Essential nutritional elements found in sugar products*

Atomic number	Element	Purpose
23	Vanadium	Growth rate.
24	Chromium	Glucose metabolism, growth.
25	Manganese	Carbohydrate metabolism, growth.
26	Iron	In hemoglobin, carbohydrate metabolism.
27	Cobalt	In vitamin B–12, antianemia.
29	Copper	Antianemia, iron metabolism.
30	Zinc	Protein metabolism, growth and healing.
34	Selenium	Protects red cells.
42	Molybdenum	Purine metabolism.
50	Tin	Growth.

of trace elements in foods and tissues; dose-effect relationships in man and animals; and possible interactions of trace elements" (*10*). Table 2 shows the toxic elements of foremost interest, including those which are usually regulated by the Food and Drug Administration and other agencies. Limits on arsenic, lead, and copper in sugar are given in the Codex Alimentarius; limits on tin, copper, and, of course mercury are given for most food products by various regulatory agencies. Thallium is a poison (in rodenticides) that has recently been in the news.

Cadmium and chromium in particular are liable to arouse public suspicion. Cadmium levels are related to blood pressure level and heart disease. With respect to chromium, very low levels rather than high are of particular interest. There have been some studies (*2*, *19*) on the relationship between chromium content of sugars (raw, refined white and brown) and serum cholesterol levels in rats. It appeared that the refined sugar diet, which was deficient in chromium, resulted in relatively high cholesterol levels, which diminished when chromium supplement was added. However, it was concluded that the results were difficult to relate to epidemiological observations.

TABLE 2.—*Toxic elements*

Atomic number	Element	Presence in sugar[1]
24	Chromium	X
28	Nickel	X
29	Copper	X
33	Arsenic	...
34	Selenium	X
48	Cadmium	X
50	Tin	X
51	Antimony	X
56	Barium	X
80	Mercury	...
81	Thallium	...
82	Lead	X

[1] X denotes presence in sugar products.

REGULATIONS

Regulations for food additives and for heavy metal contamination have been established by the Food and Drug Administration for the United States and by the international FAO/WHO organization in the Codex Alimentarius.

United States

The FDA does not have a standard of identity or guidelines for raw or refined sugars, molasses, or liquid sugars. Sugar has been on the Generally Recognized As Safe (GRAS) list since 1958 (*5*). FDA tolerances and limits are published in the FDA regulations. Guidelines are not published, and are usually the limit of the methodology

(e.g., 0.05 p/m Hg by atomic absorption spectroscopy, or less than 3 p/b by neutron activation analysis). There is a standard of identity for caramel (the listed product closest to those of the sugar industry) in its use as a color additive prepared from heat treatment of various types of sugars (*3*). The limits on heavy metals in caramel are shown in table 3.

Several heavy metals, in the form of particular salts for specific uses, are on the GRAS list (table 4).

A recent FDA survey on mercury contamination (*20*) included sugar and flour. The median contents of Hg in both sugar and flour, by neutron activation analysis, were found to be 3 p/b. There are no limits or guidelines on heavy metals in flour, but enriched flour is given a standard of identity with regard to iron and calcium, the elements in which it is enriched (*4*). The mercury limit for most foods (fish, for example) is 0.5 p/m Hg, but a limit of 0.05 p/m is advised for high-intake foods such as sugar and flour. Apparently, the FDA is considering that a limit of 0.05 p/m should be assumed for other heavy metals, unless the lower limit of detection available is above that figure.

TABLE 3.—*FDA limits on heavy metals in caramel*

Element	Parts per million
Arsenic	< 3
Lead	<10
Mercury	< .1

TABLE 4.—*Heavy metals on GRAS list*

Element	Limit
Fe	No limit as nutrient or dietary supplement.
Mn ($MnCl_2$)	Do.
Zn ($ZnSO_4$)	Do.
Si	Do.
Sn ($SnCl_2$)	Up to 15 p/m as a preservative.
Cu (CuI)	Up to 100 p/m in table salt.
Ti (TiO_2)	Up to 1,000 p/m by weight as a colorant if Pb<10 p/m, As<1 p/m, Sb<2 p/m, and Hg<1 p/m.

International

The Codex Alimentarius Commission, the international, FAO/WHO food regulatory committee of the United Nations, recommends the limits for refined sugar shown in table 5.

In the FAO/WHO Evaluation of Food Additives (*11*), the heavy metal contaminants considered are copper, mercury, and tin. Copper and tin, both essential elements, are sometimes added in small amounts for technological purposes. The Committee considered that such additions "would not significantly increase the total intake of these metals, and would therefore not be objectionable from a toxicological point of view" (*11*).

In another FAO/WHO Specification for the Identity and Purity of Food Additives (*10*), the trace elements considered are arsenic, copper, lead, mercury, tin, and zinc. The intake limits recommended for arsenic and lead (both 0.005 mg/kg body weight per day) and copper (0.05 mg/kg body weight per day) are far above the Codex limits recommended for refined sugar. No limits are recommended for tin and zinc since the usual amounts of these metals in ordinary diets are far below those necessary to produce any toxic effects. The recommendation is that the total heavy metals content for any food additive should not exceed 40 mg/kg per day.

An interesting sidelight concerns the low limits placed on arsenic. Historically, arsenic is the most famous poisonous element and has often received blame for epidemics caused by other poisons. Thus, in most manufactured foods, there is a limit of no more than 1 p/m arsenic. However, in many natural foods, this limit is far exceeded: most fish and potatoes contain more than 1 p/m arsenic, and crustaceans usually contain 3–100 p/m (*1*). Arsenic has recently been proposed as a dietary element that inhibits cancer development.

TABLE 5.—*Codex limits for metals in refined sugar*

Element	Parts per million
Arsenic	1
Lead	2
Copper	2

ANALYSES OF HEAVY METALS

The present work has two phases: a spark mass spectrometry study, which includes most of the periodic table (by Redpath and Labora-

toire d'Analyses Physique), and an atomic absorption spectrophotometry study by the Cane Sugar Refining Research Project and Southern Regional Research Center, which covers the heavy metals of particular interest.

Results from the ion spark mass spectrometry study are shown in table 6 and figure 2, which show the effect of refining the raw sugar by calcium carbonate clarification and bone char filtration. In figure 2, those elements farthest toward the upper left corner of the graph are those removed most effectively by refining.

Earlier studies were made on heavy metals in raw and refined sugars. Table 7 shows results from the current survey by all analytical methods employed, along with the ranges of these earlier results. Since the values from Cohen, Dionisio, and Drescher (*6*) are reported as oxides, the actual values will be lower than those shown here. The emission spectrograph results were in many cases confirmed by atomic absorption spectroscopy. In some of the other studies, only raw or refined sugars were examined, and the absence of a result does not mean the absence of that metal—e.g., Co.

As these results show, in most sugars, most metals are present in lower quantities in the refined sugar than in the raw. There were, however, a few cases where the reverse occurred. Increases in a metal level by refining might be due to several sources of contamination from metal machinery in the refinery, for example, dryers, screens, soldered joints, and vacuum pans. Most of the metal contaminants are components of stainless steel. A typical stainless steel composition is shown in table 8. It is also known that the ashing of a sample before analysis can introduce metal contamination, particularly if the total sample size is very large compared to the amount of ash formed. The atomic absorption technique in this survey eliminated the need for prior ashing.

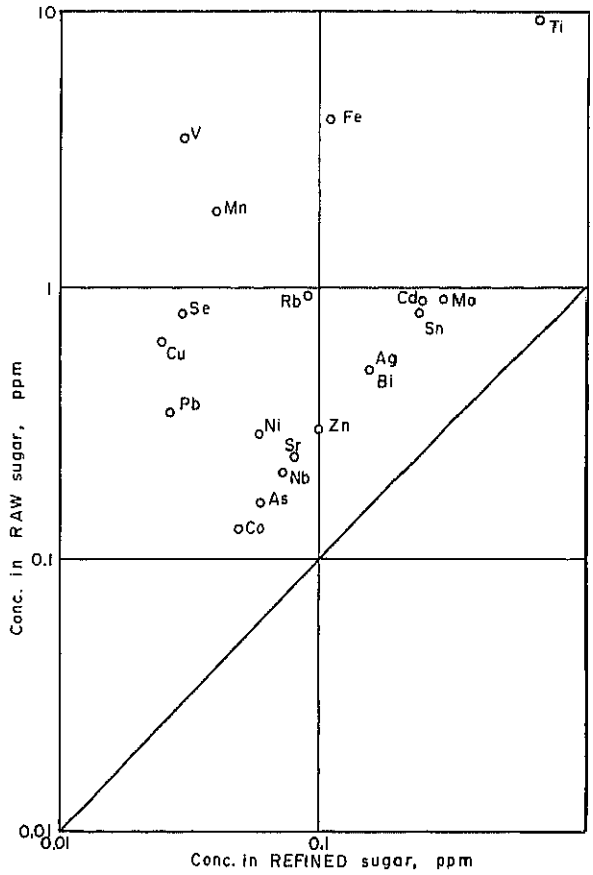


FIGURE 2.—Effect of refining on the heavy metals in sugar.

TABLE 6.—*Effect of refining process on elements in sugar*

Element	Raw (p/m)	Refined (p/m)
Boron	0.03	[1]0.01
Sodium	52.0	1.8
Silicon	62.0	([2])
Aluminum	1.8	.097
Phosphorus	21.0	([2])
Sulfur	26.0	2.7
Potassium	516.0	15.0
Calcium	94.0	4.8
Titanium	10.0	([2])
Vanadium	3.5	.03 limit
Chromium	2.1	([3])
Manganese	1.9	[1].043
Iron	4.1	.142
Nickel	.294	([2])
Copper	.51	.17
Rubidium	.925	.092
Strontium	.241	.082

[1] Detection limit.
[2] Not determinable.
[3] Masked by another substance in sample.

Considerable variation can be expected among different raws and among products of different refineries; therefore, the greater the mass of data available on metal levels, the more reliable the limits that can be placed on them.

For a basis of comparison of the levels of metals in sugar, data on metal levels in white wheat flours (*21*) are shown in table 9. These data are

TABLE 7.—*Elements in raw and refined sugar*

Element			Raw (p/m)		Refined (p/m)		
Toxic		Nut.	Current survey	Literature	Current survey	Literature	References
	Ti		10.0	3.0	<0.67L	11.9	*6*
	V	x	3.5	0.03	<0.03L	0.0028	*6*
x	Cr	x	0.1–2.1	0.06	<0.03p/bL	0.02–0.15°	*6, 19*
	Mn	x	1.8	2.1–2.4°	0.075	0.035°	*6, 9, 16*
	Fe	x	4.1	21.3°–220	0.12	4.03°	*6, 9, 16*
	Co	x	<0.13L	<1	<0.05L	.....	*9*
x	Ni	x	0.3	0.03°–<1	<0.07L	0.03–0.19°	*6, 9, 18*
x	Cu	x	0.99	3.8°–4.5	0.56	0.42	*6, 9*
	Zn	x	<0.3L	<1–8.8	<0.10L	0.62–0.97°	*6, 9, 12*
x	As		<0.16	.....	<0.06L	.....	.....
x	Sc	x	<0.7	0.01	<0.26L	0.003	*13*
	Rb		0.925	.....	<0.09L	.....	.....
	Sr		0.241	0.6	<0.08L	0.35	*6*
	Zr		<0.4L	4.2	<0.14L	0.19	*6*
	Nb		<0.211L	.....	<0.07L	.....	.....
	Mo	x	<0.9L	—	<0.3L	0.0007	*6*
	Ag		0.0006	.....	0.0009	.....	.....
x	Cd		<0.05L (AA)	.....	<0.03 p/bL	.....	.....
x	Sn	x	<0.6L	—	<0.28L	0.0007	*6*
x	Ba		<0.4L	0.09	<0.15L	0.07	*6*
x	Hg		<0.1p/b	.....	<0.1p/b	3–10p/b	*20*
x	Pb		0.07–.F	<0.2–1.4	0.03–0.3	0.02°–0.2	*6, 9, 15*
	Bi		<0.5L	.....	<0.16L	.....	.....
	Na		52.0	.....	1.8	.....	.....
	K		516.0	.....	15.0	.....	.....

° A result from reference *6* is included.
L Limit of detection of method used.
— Not present or below limit of detection.
AA By atomic absorption spectroscopy.
F By flame photometry.

TABLE 8.—*316 stainless steel composition*

Element	Percent	
C	0.08	max
Mn	2	max
Si	1	max
P	.04	max
Cr	16–18	
Ni	10–14	
Mo	2– 3	

for soft wheat flours, which tend to have a lower metal content (except for manganese) than do the hard wheat or durum wheat flours. These results are from a recent study, analyzing for this selected group of metals (plus magnesium) by atomic absorption spectroscopy. No results on iron are given, since that is added to enriched flour (*8*). The levels of these metals in refined sugar are all well below the upper limit found in these refined flours—and in several cases (Mn, Pb, Zn, Cd, and Sn) below the lower limit

TABLE 9.—*Metal levels in soft wheat flours and refined sugars*

Element	Parts per million	
	Flour	Sugar
Chromium	0.13–0.57	0.02 –0.15
Manganese	4.8 –6.4	.035– .043
Nickel	.14 – .22	.189
Copper	1.5 –1.8	.17 – .42
Zinc	.9 –4.8	.62 –1.3
Selenium	.38– .57	.003
Cadmium	.05– .08	<.06 (raw)
Tin	3.3 –8.0	.003
Lead	.66–1.53	.02 – .2

as well. When the metal levels in flours were compared to those in bread products, the levels of manganese and zinc were almost the same, indicating that flour was probably the only source of these minerals in bread. Other sources supplied a small amount of copper, and an amount of chromium almost equal to that from the flour. Nickel, tin, and cadmium levels were consider-

ably higher in bread than in flour. Since there is very little sugar compared to flour in bread and since the metals found at higher levels in bread are at very low levels in sugar, bread rates much higher in both beneficial and toxic heavy metals than does sugar.

The quantitative data from the current survey and from earlier work indicate that the sugar refining industry has no serious problems with heavy metal contamination in refined sugar.

ACKNOWLEDGMENT

The authors thank V. W. Tripp and N. M. Morris of the Cotton Properties Laboratory, Southern Regional Research Center, Agricultural Research Service, for their work in obtaining the atomic absorption spectrophotometry data.

REFERENCES

(1) Anonymous. 1966. Many trace elements affect animal nutrition. Chem. Eng. News. 44: 48–50.
(2) ———. 1969. Sugar, chromium and serum cholesterol. Nutr. Rev. 27: 185–186.
(3) Code of Federal Regulations. 1972. Caramel. Title 21, Food and Drugs. Chap. 1. Food and Drug Administration. Subsection 8.303.
(4) ———. 1972. Enriched flour identity. Title 21, Food and Drugs. Chap. 1. Food and Drug Administration. Subsection 15.10.
(5) ———. 1972. Substances that are generally recognized as safe. Title 21, Food and Drugs. Chap. 1. Food and Drug Administration. Subsection 121.-101.
(6) Cohen, M. A., Dionisio, O. G., and Drescher, S. J. 1970. The isolation and characterization of certain impurities responsible for quality problems in refined cane sugar. Sugar Ind. Tech. 24: 123–165.
(7) Frieden, E., 1972. The chemical elements of life. Sci. Am. 226: 52–60.
(8) International Commission for Uniform Methods of Sugar Analysis. 1970. Subject 3. Fifteenth Session: 22.
(9) Iwasa, N. 1967. Polarographic determination of inorganic nonsugars in sugars. I. Determination of heavy metals. Proc. Res. Soc. Jap. Sugar Refineries. 19: 1–8.
(10) Joint FAO/WHO Expert Committee on Food Additives. 1967. Specifications for the identity and purity of food additives and their toxicological evaluation. Tenth Report.
(11) ———. 1971. Specifications for the identity and purity of food additives and their toxicological evaluation. Fourteenth Report.
(12) Mee, J. M. L., and Hilton, H. W. 1969. Rapid direct analysis of zinc in sugar products and molasses. J. Agric. Food Chem. 17: 1398–1399.
(13) Morris, V. C., and Levander, O. A. 1970. Selenium content of foods. J. Nutr. 100: 1383–1388.
(14) Nunez Triana, G. 1969. Spectrophotometric determination of Ca, Mg, Fe, Mn and Cu in cane molasses. Sobre Deriv. Cana Azucar 3: 31–38.
(15) Plews, R. W. 1967. Polarographic determination of lead and copper in refinery products. Int. Sugar J. 69: 291–293.
(16) Rodriquez, A. A. 1961. Spectrographic study of the metallic composition of cane juice. Inst. Cubano Invest. Tecnol. Bol. Inf. 5: 3–5.
(17) Rodriguez, M. L., and Nunez, G. 1970. Contents of some trace elements in final molasses from Cuban sugar cane. Bol. Of. Asoc. Tec. Azucar Cuba 25: 6–12.
(18) Schlettwein-Gsell, O., and Mommsen-Straub, S. 1971. Trace elements in foods. 5. Nickel. Int. Vir-Ern. Forsch. 41: 429–37.
(19) Schroeder, H. A. 1969. Serum cholesterol and glucose levels in rats fed refined and less refined sugars and chromium. J. Nutr. 97: 237–242.
(20) Tanner, J. T., Friedman, N. H., Lincoln, D. N., Ford, L. A., Jaffee, M. 1972. Mercury content of common foods determined by activation analysis. Science 177: 1102–1103.
(21) Zook, E. G., Greene, F. E., and Morris, E. P. 1970. Nutrient composition of selected wheats and wheat products. VI. Distribution of manganese, copper, nickel, zinc, magnesium, lead, tin, cadmium, chromium and selenium as determined by atomic absorption spectroscopy and colorimetry. Cereal Chem. 47: 720–731.

DISCUSSION

R. S. PATTERSON (California and Hawaiian): You show some results on cane molasses. Did you do any testing of soft or brown sugars?

P. POMMEZ: No. However, I want to add a few remarks on our results regarding the concentration of some metals in the molasses. Supposing 100 lb of raw sugar at 98° purity yields 3.3 lb molasses at 45° purity, the concentration of any element in molasses to that of the same element in the raw should be in a theoretical ratio of 66. An experimental ratio can be derived from

$$\frac{\%\text{ element in molasses (on solids)}}{\%\text{ element in raw}-\%\text{ element in refined}}$$

A value close to 66 would mean that all the element removed from the raw has been concentrated in the molasses. A smaller value would indicate that the element removed does not appear in molasses but has been retained at some stage of the refinery process. We give in the table shown below figures relative to Na, Cu, and Pb.

Element	Element on solids (p/m)			Theo-retical ratio	Experi-mental ratio
	Molasses	Raw	Refined		
Na	3,300	52	1.8	66	65.8
Cu	3.3	.64	.13	66	6.6
Pb	.37	.069	.027	66	8.8

It is noteworthy that the sodium which is not affected by any of the psysicochemical treatment appears in the molasses at a ratio very close to the theoretical value, whereas the values for Cu and Pb suggest that during the refining process the heavy metals are eliminated to a large extent, probably through adsorption on bone char.

G. W. MULLER (Kerr-McGee): Some metal contamination could come from stainless steel. I wonder, if you made some of these tests on some historic samples, such as from the 1920's, when there was comparatively little stainless in the refinery, and most of the equipment was made from iron or copper, if you would find some of these other metals in your product. A look at an analysis on stainless steels shows that the liquors are contacting a far greater variety of metals today than in the old days. Copper is certainly removed in refining—we don't have to worry about it.

P. POMMEZ: No, we didn't try old samples. You are probably correct; some metals may indeed come from the stainless steel.

K. R. HANSON (Amstar): About a year ago, Dr. H. A. Schroeder of Dartmouth claimed that chromium was an essential element in the prevention of heart attacks and that sugar did not contain enough chromium.[1]

M. A. CLARKE: Yes, chromium would be one element of benefit in refined sugars.

N. H. SMITH (California and Hawaiian): Have you had occasion to look at halogens at all?

P. POMMEZ: In this particular study, we did not pay special attention to the halogens. However, the fate of chloride in the process has been the subject of intensive investigation at Redpath Sugars, and a paper was given at the 1970 Technical Session in Boston,[2] focusing on the possibility of using chloride as a measurement of affination efficiency, and even of the whole refinery process.

J. A. WATSON (Tate & Lyle): These tests you have done are on cane raws in a bone char refinery. Have you any information on cane raws in a granular carbon refinery?

M. A. CLARKE: No, we used only one raw and one refined sugar to make the analyses. Some of the literature results may be from other types of refineries; we do not have data on all the sources of those sugars. We plan to do future work on raws in a granular carbon refinery?

P. POMMEZ: I would like to point out that there are difficulties in sampling both raw and refined sugars, if you want to follow elements through the refining process. You have to try to use the same raw sugar for some time in the refinery, which is difficult in our case as we use sugars from different origins which are mixed up in the raw shed; then, you take a sample of a few pounds of refined, which has been processed from the raw of a few days before. The same problems happen with the molasses, to an even greater degree. So, our figures are average figures, not for any specific raws.

M. C. BENNETT (Tate & Lyle): Since we are all faced with the problem of measuring these metals for one reason or another, I would be grateful for a little more on the experimental side. You referred very briefly to a polarographic method. Could we have a little more information about that method?

P. POMMEZ: The polarographic technique used was differential pulse anodic stripping. That technique proceeds in two steps: First, a portion of the metal ions in solution is deposited electrolytically into a hanging mercury electrode to preconcentrate the metals in the form of a dilute amalgam; second, the metals in the amalgam are reoxidized to the component ion and their concentration is determined by measuring the current which flows as the material is "stripped" into the solution. The combination of anodic stripping voltammetry with the pulsed excitation used in differential pulse polarography allows one to obtain extreme sensitivities using short deposition times (2 to 5 minutes) and yields curves (peak form) that are easy to interpret. The instrument used was a Princeton Applied Research model 175 polarographic analyzer.

[1] Schroeder, H. A. 1969. Serum cholesterol and glucose level in rats fed refined and less refined sugars and chromium. J. Nutr. 97: 237–242.

[2] Pommez, P., and Stachenko, S. 1970. Use of chloride electrodes in refinery control. Proc. 1970 Tech. Sess. Cane Sugar Refining, pp. 82–102.

FLUORESCENCE IN COMMERCIAL SUGARS

By Frank G. Carpenter and James H. Wall[1]
(Presented by Frank G. Carpenter)

ABSTRACT

Instrumental corrections were applied to fluorescence measurements in commercial sugars. The resulting, corrected, equal-energy fluorescence spectra showed four principal peaks which were related to refining processes. Measurements of fluorescence show promise as a useful control in cane sugar refining and could be more informative than the color measurement that is presently used.

INTRODUCTION

A preliminary report from this laboratory on the fluorescence of sugars (*9*)[2] indicated that fluorescence might be a useful optical measurement for evaluating minor constituents in sugars. The preliminary work did not consider any instrumental corrections, and those results could only be interpreted in a broad sense. This report considers several corrections and these results reveal a finer structure which has interesting interpretations.

THEORY

By definition, fluorescence occurs when light is absorbed at one wavelength and then reemitted at a longer wavelength. Using I_A for the intensity of the light absorbed and I_F for the fluorescent intensity, the definition can be expressed mathematically in the form

$$I_F = QI_A, \qquad (1)$$

where Q is the constant of proportionality, also known as the quantum yield.

At this point it is necessary to consider the units. The theoretician and the physicist would measure all of the light intensities in quanta because these are the more fundamental units; however, the early workers in optics used energy units for evaluating intensities, and the instrument makers have perpetuated these units. Since we are more interested in practice than in theory, we will continue with energy units. The fact that the excitation is at a different wavelength than the emission is what makes the difference between the two units, because a quantum of light, Q_L, at a shorter wavelength, λ, represents a higher energy, E, than does a quantum at a longer wavelength. The relation is expressed

$$Q_L = E\lambda. \qquad (2)$$

In this paper, the I_F and I_A in equation 1, which are at different wavelengths, are expressed in energy units, so the flourescent spectra obtained are for equal excitation energy instead of equal-excitation quantum flux.

The idea of an equal-energy spectrum is fine in theory, but it must be measured with a real instrument, not a theoretical instrument, and real instruments are not inherently of equal energy over the whole spectrum. The light source is not of equal energy over its wavelength range, the energy transmission of the monochromators is wavelength dependent, the response of the photodetector is not linear in energy of different wavelengths, and the attenuation of light energy within the sample itself is also dependent upon wavelength. Therefore, corrections must be made for all these factors (and more) to obtain a true equal-energy fluorescent spectrum from a real instrument.

It is not always necessary to have an equal-energy spectrum; if for instance, the same instrument is always to be used, as in control work. But, if comparisons are to be made between instruments or laboratories, or if some fundamental interpretation of the results is required, then

[1] Research leader and chemist, Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, New Orleans, La. 70179.

[2] Italic numbers in parentheses refer to items under "References" at the end of this paper.

an equal-exciting energy fluorescent spectrum is required to put the results on a comparable basis. Instruments have been designed to automatically (*4*) make all these corrections, which is one solution to the problem, but an expensive one. However, in this age of computers, it is probably easier to use a simpler instrument and calculate the correction factors.

INSTRUMENT GEOMETRY

Fluorescence is measured by an instrument which has the configuration shown in figure 1. The excitation monochromator selects one wavelength from the source which irradiates the sample. The emission monochromator selects one fluorescent wavelength for detection.

In the sample cell in figure 2, the exciting beam is defined by a slit system and does not fill the entire sample cell, but only a narrow path (not necessarily centered) through the cell. The active fluorescing element within the sample cell is only that portion which is both excited by the entrance beam and also seen by the emission optical system.

In this active element within the sample cell

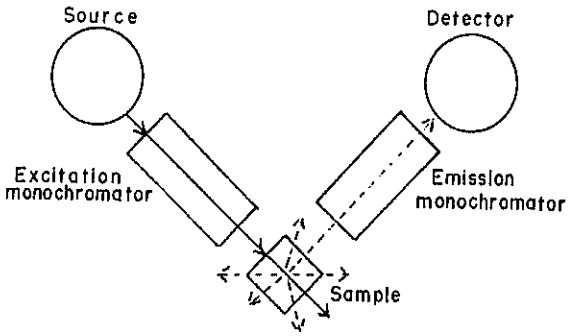


FIGURE 1.—General configuration of instruments for measuring fluorescence.

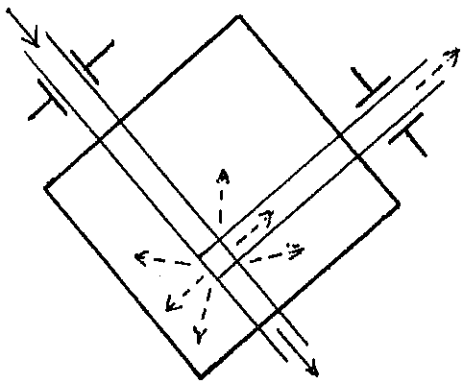


FIGURE 2.—Light paths within the sample cell.

(fig. 3), the intensity of the entrance beam will be temporarily defined as I_0, the transmitted beam as I_T, and the fluorescent intensity as I_F, all in relation to the active fluorescing element.

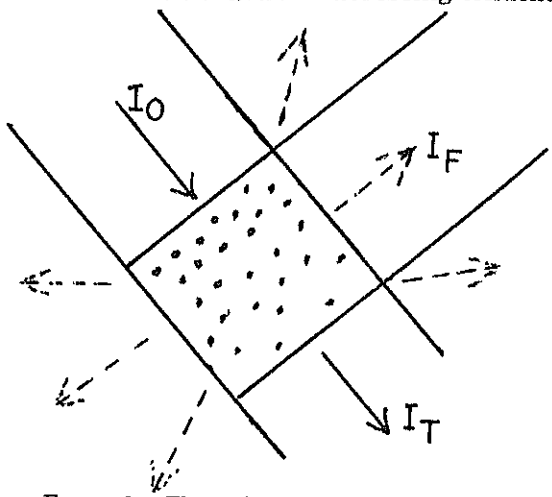


FIGURE 3.—The active fluorescing element.

The absorbed intensity, I_A, in equation 1 is not measured, but rather the incident intensity, I_0, and the transmitted intensity, I_T, are measured. On the assumption that all the light that is not transmitted is absorbed,

$$I_A = I_0 - I_T. \tag{3}$$

This assumption is not strictly correct for sugar solutions (*8*), because some light is scattered. This error is small for dark-colored solutions but can easily be 50 % for the light-colored solutions. The effect of this error is to make the corrected fluorescence for light-colored solutions too low by a factor of up to 50 % when compared to dark-colored solutions. Since scattering is greater at shorter wavelengths, the corrected fluorescence will also be too low at shorter wavelengths, which will result in shifting the fluorescence peak slightly toward the red. These errors due to scattering are not considered in this paper. Insertion of equation 3 into equation 1 gives

$$I_F = Q(I_0 - I_T). \tag{4}$$

Definition of the transmission across the active fluorescing element in the usual way gives

$$T = I_T / I_0. \tag{5}$$

Equation 4 now becomes

$$I_F = QI_0(1 - T). \tag{6}$$

At this point T is expressed as

$$T=10^{\log T} \tag{7}$$

because, when so expressed, it becomes an exponential and thus can be expanded in the exponential series:

$$T=10^{\log T}=1+\frac{2.3\log T}{1!}+\frac{(2.3\log T)^2}{2!}+\frac{(2.3\log T)^3}{3!}+\ldots+\frac{(2.3\log T)^n}{n!}+\ldots. \tag{8}$$

Equation 8 substituted into equation 6 gives

$$I_F=QI_0\ 1-1-2.3\log T-\frac{(2.3\log T)^2}{2!}-\frac{(2.3\log T)^3}{3!}-\ldots-\frac{(2.3\log T)^n}{n!}-\ldots. \tag{9}$$

In equation 9 the 1's cancel out and (—2.3 log T) can be factored out, giving

$$I_F=QI_0\ (-2.3\log T)\ 1+\frac{2.3\log T}{2!}+\frac{(2.3\log T)^2}{3!}+\ldots+\frac{(2.3\log T)^{n-1}}{n!}+\ldots. \tag{10}$$

The last term (which contains the series) is a correction for the lack of transmission across the active fluorescing element shown in figure 3. It accounts for the fact that some of the incident light is absorbed in passing through the active fluorescing element; thus, the backside receives less excitation and fluoresces less. Figure 3 illustrates this with fewer fluorescing centers on the backside of the active fluorescing element. This *transmission correction within the active fluorescing element* is written

$$T_c=1+\frac{2.3\log T}{2!}+\frac{(2.3\log T)^2}{3!}+\ldots+\frac{(2.3\log T)^{n-1}}{n!}+\ldots. \tag{11}$$

This is a function of the transmission across the active fluorescing element only, and has the values given in table 1.

Usually the transmission across the active fluorescing element is quite high, approaching 1, so this is a small correction, but if a computer is being used this simple calculation is also included.

In equation 10, there is another term containing transmission (which is the primary effect of transmission), which can be expressed

TABLE 1.—*Transmission correction within the active fluorescing element*

T	T_c	T	T_c
1.0000	1.00000	0.2500	0.54101
.9500	.97479	.2000	.49707
.9000	.94912	.1500	.44805
.8500	.92297	.1000	.39087
.8000	.89628	.0500	.31712
.7500	.86901	.0250	.26431
.7000	.84110	.0125	.22535
.6500	.81247	.00625	.19581
.6000	.78305	.003125	.17282
.5500	.75271	.001563	.15452
.5000	.72135	.000781	.13966
.4500	.68878	.000391	.12738
.4000	.65481	.000195	.11706
.3500	.61915	.000098	.10828
.3000	.58141	.000049	.10072

$$-\log T=abc. \tag{12}$$

In this equation, b is the cell depth within the active fluorescing element for the exciting beam; c, the concentration of the light absorbing (and fluorescing) species; and a, the constant of proportionality, called absorptivity index or extinction coefficient. In this way, the concentration of the fluorescing material enters the equation for fluorescence. Inserting equations 11 and 12 into equation 10 gives

$$I_F=QI_0 2.3abcT_c. \tag{13}$$

The I_F and I_0 in equation 13 refer to the theoretical values at the active fluorescing element as shown in figure 3. In reality the values at the entrance and exit of the whole sample cell must be considered, as shown in figure 4. The intensity of the exciting beam in figure 4 is decreased by an amount T_S in traversing from the cell entrance to the active fluorescing element. Likewise the fluorescent light is decreased by an amount T_R in reaching the cell wall. The equation then becomes

$$I_F=2.3QabI_0T_ScT_cT_R. \tag{14}$$

T_S and T_R are transmissions of the test solution at the source and response wavelengths for the path-length distance of the geometry of the instrument.

Transmission is ordinarily measured in, or calculated to, a 1-cm cell depth. The above T_S and also the T used in calculating T_c are related

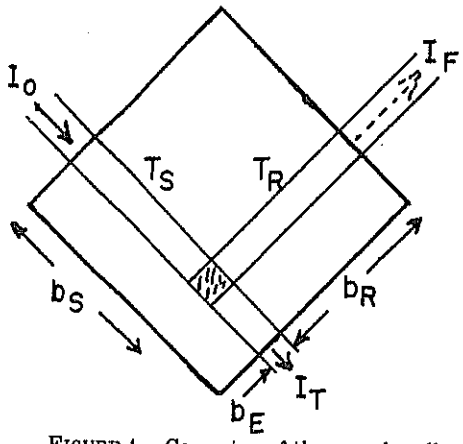


FIGURE 4.—Geometry of the sample cell.

to the usual transmission, T, by the following relations:

$$\frac{-\log T_R}{b_R}=\frac{-\log T}{1};\quad \frac{-\log T_S}{b_S}=\frac{-\log T}{1};$$

$$\frac{-\log T_E}{b_E}=\frac{-\log T}{1}. \tag{15}$$

The T_S and T_R calculated by equations 15 are termed T_{sbs} and T_{RbR}, whereas the transmission correction calculated from the series in equation 11 using the T_E from equation 15 is termed T_{cE}. Note that all the corrections so far can be evaluated from a transmission curve for the sample solution. There must be at least some transmission over the entire wavelength range of interest, because if T goes to zero then so does I_F. The equation then becomes

$$I_F=2.3QabI_0T_{sbs}cT_{cE}T_{RbR}. \tag{16}$$

I_0 and I_F in equation 16 refer to the values at the sample cell whereas ultimately the values at the source lamp and at the phototube detector (fig. 5) are the values to be considered.

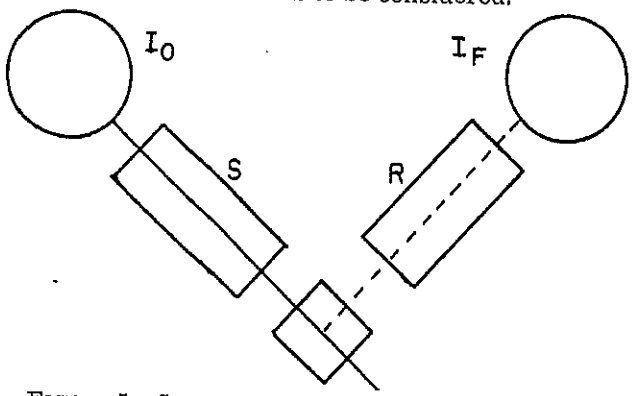


FIGURE 5.—Source and response factors in complete fluorescence instrument.

S represents the relative output of the lamp including any attenuation of the monochromator. R is the relative response of the detector, including the emission monochromator. Inclusion of these terms in the equation completes all the instrumental corrections.

$$I_F=2.3QabI_0ST_{sbs}cT_{cE}T_{RbR}R. \tag{17}$$

STANDARD REFERENCE

There remains only the problem of evaluating I_0 which is many orders of magnitude greater than I_F and beyond the linear range of the usual photodetectors. The method of comparison with a standard was used and the standard chosen was the Raman peak of water excited at 350 nm and emitted at 400 nm. This method also has the advantage of cancelling out certain geometric factors which have not been discussed. The Raman peak of water, I_W, is measured by placing water in the sample cell. It is proportional to the same I_0 which is decreased by the same source factor S but, in this case, for the fixed wavelength 350 nm, and so is written S_{350}. The Raman emission is measured by the same photodetector, but always at 400 nm, and so is written R_{400}. It is also proportional to the same active element path length b as appeared in the fluorescence measurement. The Raman peak for water is therefore

$$I_W=kbI_0S_{350}R_{400}. \tag{18}$$

No other corrections for within the sample cell appear because water has 100% transmission at these wavelengths. The Raman peak of water is substituted as a measure of the lamp intensity by eliminating I_0 between equations 17 and 18:

$$I_F=\frac{2.3\,Qa}{k}I_W\frac{S}{S_{350}}T_{sbs}cT_{cE}T_{RbR}\frac{R}{R_{400}}. \tag{19}$$

Incidentally, the path length b's also disappear. Placing all of the measurable terms on the same side of the equation results in the following working equation:

$$\frac{I_F}{I_Wc}\,\frac{S_{350}}{S}\,\frac{R_{400}}{R}\,\frac{1}{T_{sbs}T_{cE}T_{RbR}}=\frac{2.3Qa}{k} \tag{20}$$

The constants in this equation, a and Q, are the absorption index and quantum yield, respectively, of the substance that is absorbing and emitting the light, whereas k is the size of the Raman peak of water to which everything is being referred. This equation says that the fluor-

escent intensity as a multiple of the Raman peak of water, per unit concentration, corrected for all the various factors, is the way to express the fluorescence and that it should be a constant for any given material.

The assumptions and necessary conditions that have gone into this equation are as follows:

1. There is negligible scattering as compared to absorption of light.

2. The Raman peak of water is measured with the same instrument geometry as the fluorescence.

The second condition would normally be present but the first is a serious limitation on the accuracy which should be considered.

CONCENTRATION

The problem of evaluating the concentration, c, of an unknown component in sugar remains the same in fluorescence as it is in colorimetry. The concentration of sugar, C_S (or total solids), is used instead and the results will be proportional to the relative concentration of unknown component in the sugar.

$$\frac{I_F}{I_W C_S}\ \frac{S_{350}}{S}\ \frac{R_{400}}{R}\ \frac{1}{T_{SbS}T_{CE}T_{RbR}} = \frac{2.3Qa}{k}\ \frac{c}{C_S} \quad (21)$$

DESCRIPTION OF INSTRUMENT

The fluorescence instrument used was the Fluorispec fluorescence spectrophotometer, model SF-1.[3] The light source was a xenon lamp, the monochromators were the double-grating type, and the photodetector was a 1P21 photomultiplier

The slit system gave a light beam 1 mm wide in the sample cell (b_E=0.1 cm in equation 15), and the excitation path length within the sample cell before reaching the active fluorescing element was 8 mm. The emission beam path length within the sample cell was also 8 mm. In equation 15, b_S and b_R were therefore both 0.8 cm.

The wavelength scan of the instrument was connected to the X-axis of an X-Y recorder and the output of the photomultiplier connected to the Y-axis. The circuit contained a range change network of $\times$10 and $\times$100. These ranges were checked and found to be linear and reliable to better than 1%. The fluorescent light intensities were read in arbitrary units off the chart. It is most important to read the Raman peak of water in the same units, considering the range.

[3] Manufactured by Baird-Atomic, Inc., $8000.

Evaluation of Source and Response

The evaluation of source, S, and response, R, functions was obtained by use of a standard lamp. There is no method by which these functions can be self-evaluated within the instrument. One technique for evaluating these functions is to masure the fluorescnce of a known material (*1–3, 5–7, 10*); however, there remains the problem of the reliability of the fluorescence data on a sufficient number of materials to adequately cover the required range. The most straightforward evaluation scheme uses a standard lamp in the configuration shown in figure 6.

The lamp used was a 1000-watt quartz halogen tungsten coiled-coil filament incandescent lamp. Its calibration was traceable to the National Bureau of Standards.[4] This type of lamp is noted for its long life and constant light output over its entire life. Being an incandescent lamp, it should follow the Planck radiation law, and indeed 15 calibrated points covering the range 270–750 nm agreed with the Planck law with a coefficient of variation of 1.8%. The Planck law with a color temperature of 3167.1° K was therefore used to interpolate between calibrated points. The use of the standard lamp required an accurate control and knowledge of the lamp current of 8.30 amperes AC. An ammeter with 0.5% accuracy[5] was used for this purpose. The ratio between the

[4] Calibrated by Optronic Laboratories, Inc., 7676 Fenton Street, Silver Spring, Md., $250.

[5] Weston model 433, $160.

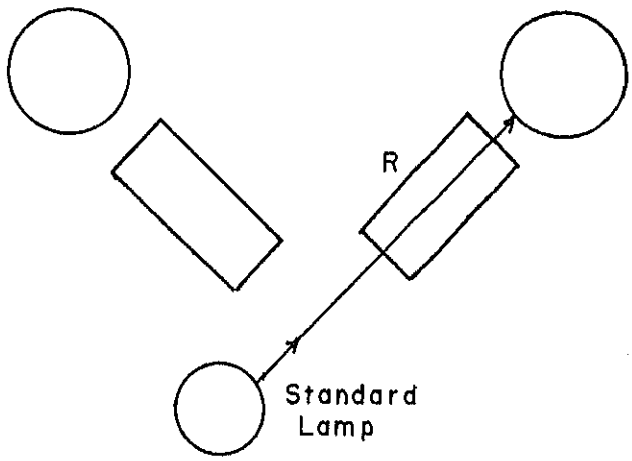


FIGURE 6.—Configuration of instrument for evaluating response.

measured output of the photodetector and the known intensity of the standard lamp was the required response factor, R, of the emission monochromator and phototube combined. This ratio is graphed in figure 7 and given numerically in table 2. Only relative, not absolute, values at the different wavelengths were required because the Raman peak of water gave an absolute value to which everything was referred. The falloff in response at wavelengths above 600 nm is a property of the S4 photocathode of the 1P21 photomultiplier. The falloff in response at wavelengths below 300 nm results from the combined effect of the phototube and the grating monochromator.

The source was calibrated with the instrument configuration shown in figure 8.

Magnesium oxide is the recognized standard of 100 % reflectance, and by reflecting the source into the already calibrated response system the source could be evaluated. The results are given in figure 7 and table 2. The falloff in intensity below about 250 nm is a property of the monochromator. The fine structure above 400 nm is a property of the xenon arc which is undesirable but cannot be overcome on this instrument.

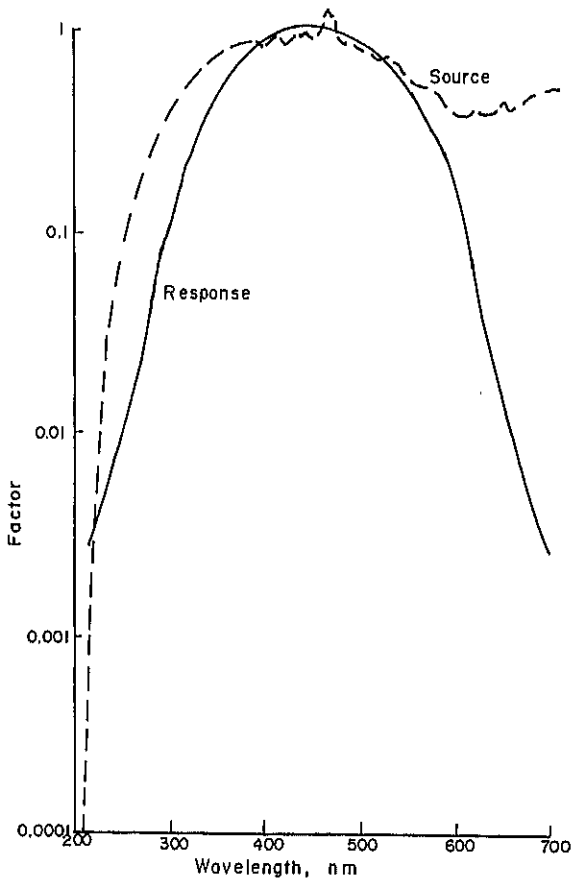


FIGURE 7.—Source and response factors for the instrument used.

TABLE 2.—*Response and source factors*

Wavelength (nm)	R	S	Wavelength (nm)	R	S
220	0.0024	0.0022	470	0.9595	1.2908
230	.0036	.0105	480	.9181	.8414
240	.0053	.0374	490	.8726	.7822
250	.0078	.0726	500	.8269	.7323
260	.0126	.1133	510	.7793	.6828
270	.0199	.1709	520	.7388	.6780
280	.0369	.2350	530	.6920	.6947
290	.0559	.2953	540	.6172	.6333
300	.0864	.3607	550	.5220	.5659
310	.1364	.4170	560	.4290	.5132
320	.1854	.5443	570	.3490	.4942
330	.2717	.5943	580	.2913	.4661
340	.3528	.6761	590	.2379	.4474
350	.4494	.7104	600	.1686	.3810
360	.5255	.7682	610	.0982	.3361
370	.6268	.7904	620	.0568	.3551
380	.7354	.8234	630	.0363	.3534
390	.8248	.8454	640	.0239	.3444
400	.8914	.9160	650	.0146	.4121
410	.9291	.8433	660	.0094	.3673
420	.9365	.8993	670	.0061	.3897
430	.9586	.8614	680	.0042	.4292
440	.9774	.8973	690	.0031	.4653
450	1.0000	1.0000	700	.0023	.4638
460	.9891	.8997			

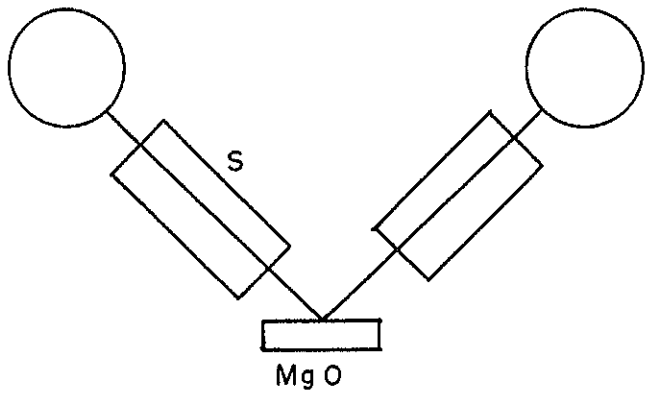


FIGURE 8.—Configuration of the instrument for evaluation of source.

MEASUREMENT AND CALCULATIONS

All sugar solutions were measured at pH 10. It is well recognized that pH is an important factor in the fluorescence of sugars, but it is beyond the scope of this report.

Each solution to be measured for fluorescence was first checked for transmission above about 5% for all wavelengths down to 250 nm. If the transmission did not go to zero (less than 1%), then a complete transmission curve was run over the wavelength range 700 nm to 250 nm in a recording spectrophotometer (Beckman DB). Solutions that did not pass the transmission test were diluted until they did.

The sample was then placed in the fluorescence instrument and emission curves run at about 5 to 9 fixed excitation wavelengths that covered the range of interest. Finally, the Raman peak of water was obtained for reference without changing any of the instrument controls. A typical set of curves for the raw data is shown in figure 9. The curves obtained were digitized at 10-nm intervals.

The following calculation was performed by the computer:

$$F = \frac{I_F S_{350} \quad R_{400}}{I_W c \exp\,(0.8 \ln T_{EX})\, S_{EX} T_C \exp\,(0.8 \ln T_{EM})\, R_{EM}}$$

where subscript $_{EX}$ refers to excitation wavelength,
subscript $_{EM}$ refers to emission wavelength,
and T_C is the solution for equation 11 using exp $(0.1 \ln T_{EX})$ for T.

In the usual run, this calculation was repeated for each of about 30 points on each of about 7 curves. The resulting corrected fluorescences for the same raw data as shown in figure 9 are shown in figure 10. It is evident that the corrections are not trivial.

This method of expressing the results is not easy to interpret. A more satisfactory plot is emission wavelength versus excitation wavelength for constant intensity of fluorescence. This is achieved by interpolating the curves of figure 10 at constant fluorescence as shown by the dotted line. Interpolation was made both along the emission curves and between the curves of different excitation. The resulting plot is a contour chart of equal fluorescence lines which are closed curves surrounding the fluorescence mountain. The curve plotter on the computer was used to plot these fluorescence contour charts. The fluorescence levels chosen were a quasi-logarithmic series: 100, 60, 40, 25, 15, 10. These were multiplied by an appropriate power of 10 to cover the range of the data. Note that there is a factor of about 1.5 between contours and 5 contours is exactly a factor of 10.

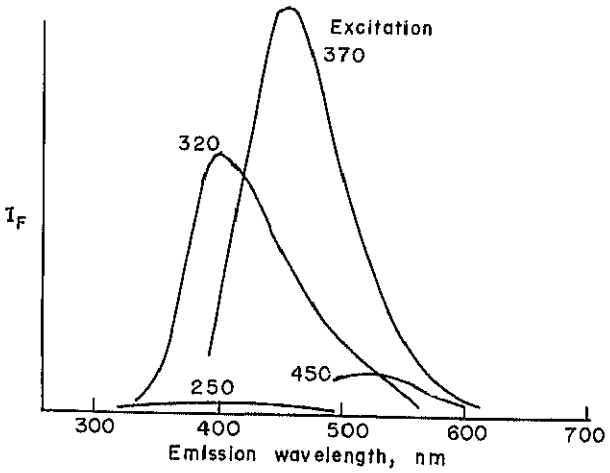


FIGURE 9.—Typical fluorescence data for refined sugar, uncorrected.

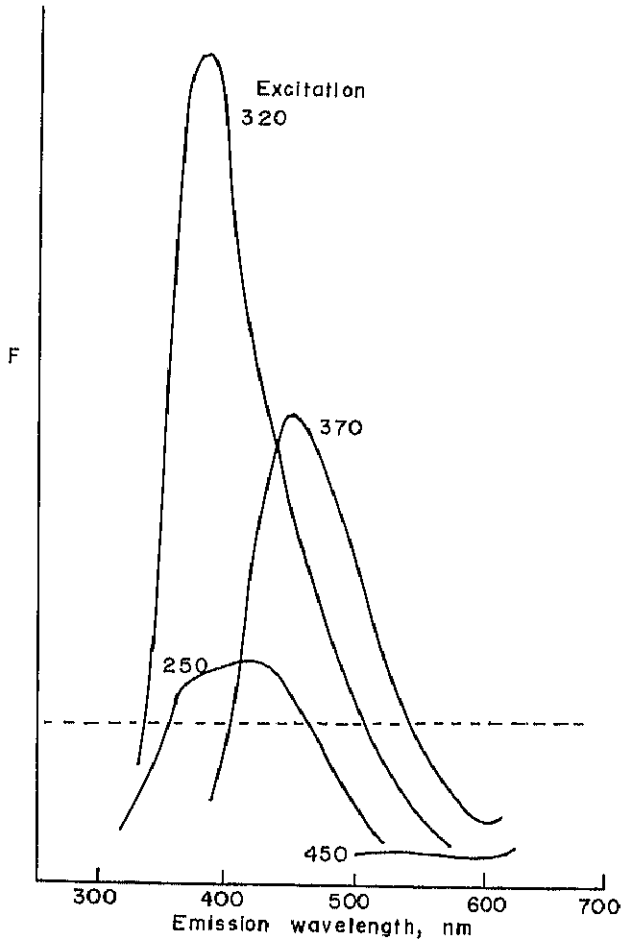


FIGURE 10.—Data from figure 9 (fluorescence data for refined sugar), corrected.

The advantage of this type of fluorescence diagram is that it shows at a glance the position of the fluorescence peak in terms of excitation and emission wavelengths and relegates to secondary importance the height of the peak as the parameter. It is the position of the peak that is related to the nature of the fluorescent substance. The main purpose of all the corrections that have been applied was to obtain the correct position of the peak. Ignoring the light scattering has left error in the height of the peak, so this feature cannot be scrutinized too closely. The position of the peak is hereafter designated (excitation, emission), for example, (360, 430). The height of the peaks will be understood to be in multiples of the Raman peak of water, and per unit concentration of sugar in sugars, or per unit concentration of known materials.

RESULTS

The fluorescence diagrams for two typical molasses are shown in figures 11 and 12. The bone char refinery molasses in figure 11 is dominated by a major peak of over 100,000 at (360,-430). However, there is a long ridge running toward (250,430). In addition there is a suggestion of some little bump at about (400,600). In the

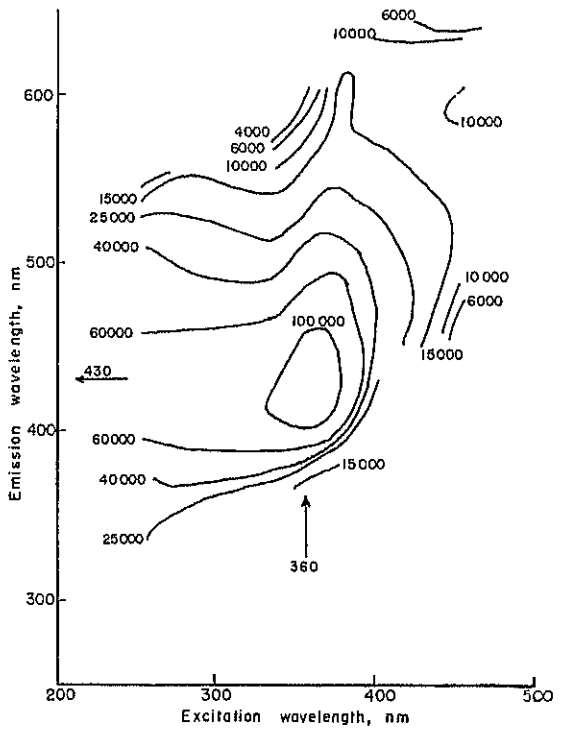


FIGURE 11.—Fluorescence diagram of bone char refinery molasses.

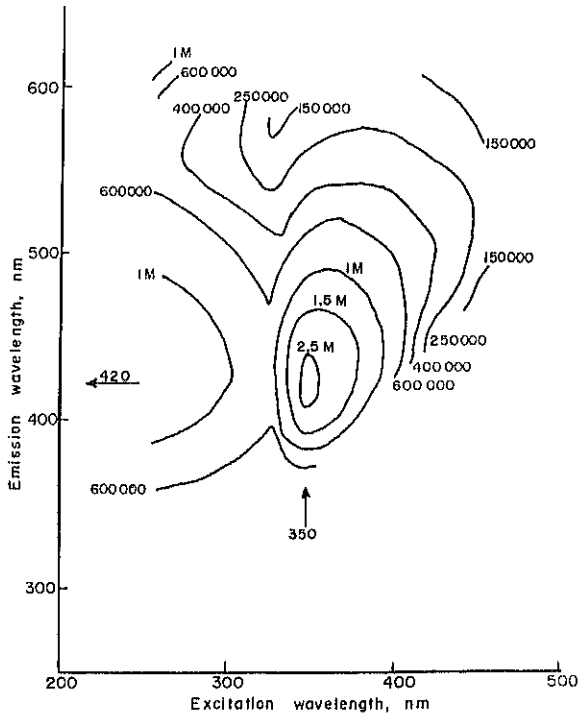


FIGURE 12.—Fluorescence diagram of raw house molasses.

raw house molasses, the main peak at (350,420) is even higher, and it separated slightly from that ridge which has now become a bump at (250,420). The main peak is also stretched out toward (400,600) even though no separate peak appears.

Figures 13 through 16 show the fluorescence diagrams of typical raw sugars. In each of these the main peak is similarly situated at about (350,420). The height is 1,000 times less than in molasses. The secondary peak, near (250,420), is only suggested. If it is there at all, it must be just out of range. The peak at (400,600) shows up well and is especially evident in the Argentine raw shown in figure 15. A small sharp peak that is sometimes higher than the main central peak appears at (280,310). It is possible that in molasses these last two peaks were overshadowed by the main central peak.

Some additional information about the substances that cause these peaks can be obtained from the fluorescence diagram of molasses browning polymer shown in figures 17 and 18. These substances were obtained from W. W. Binkley of the New York Sugar Trade Laboratory. The nondialyzable part of the molasses colorant shown in figure 17 had all the peaks present, whereas the dialyzable part showed a con-

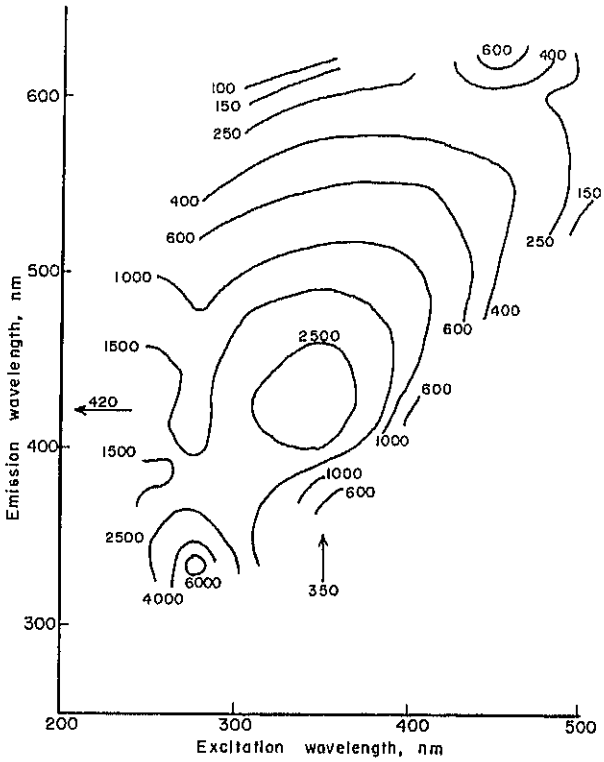


FIGURE 13.—Fluorescence diagram of Brazilian raw sugar.

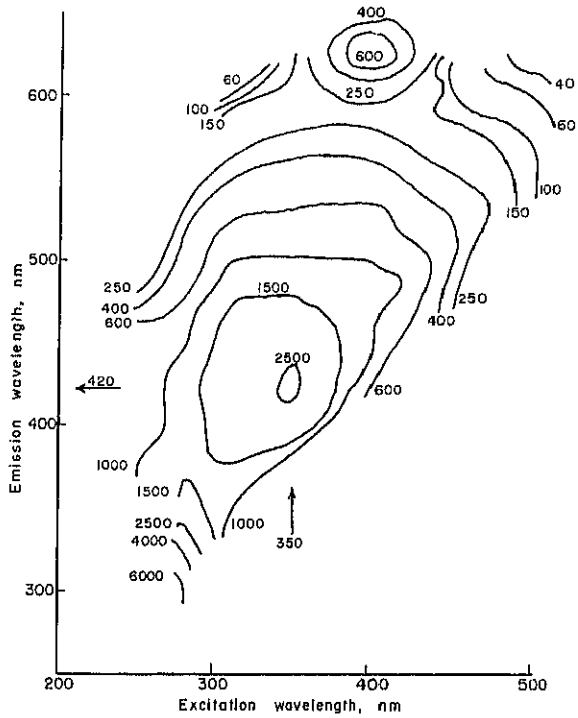


FIGURE 15.—Fluorescence diagram of Argentine raw sugar.

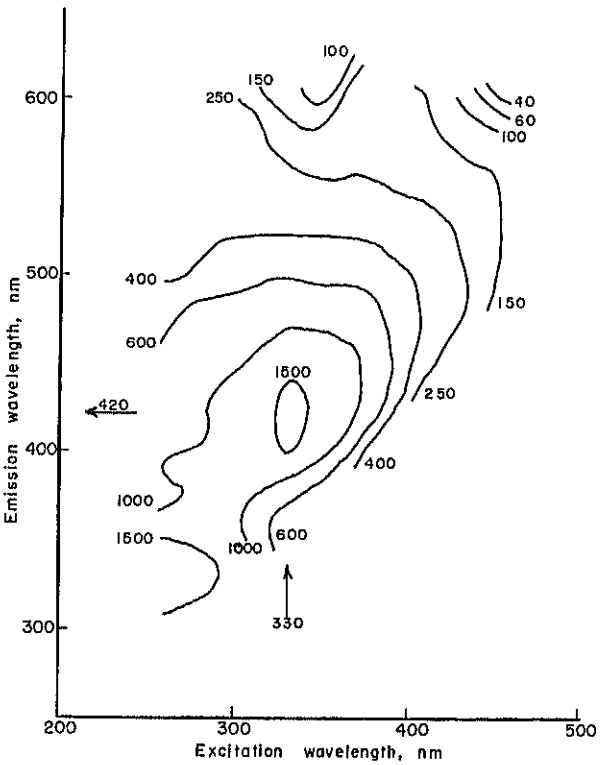


FIGURE 14.—Fluorescence diagram of South African raw sugar.

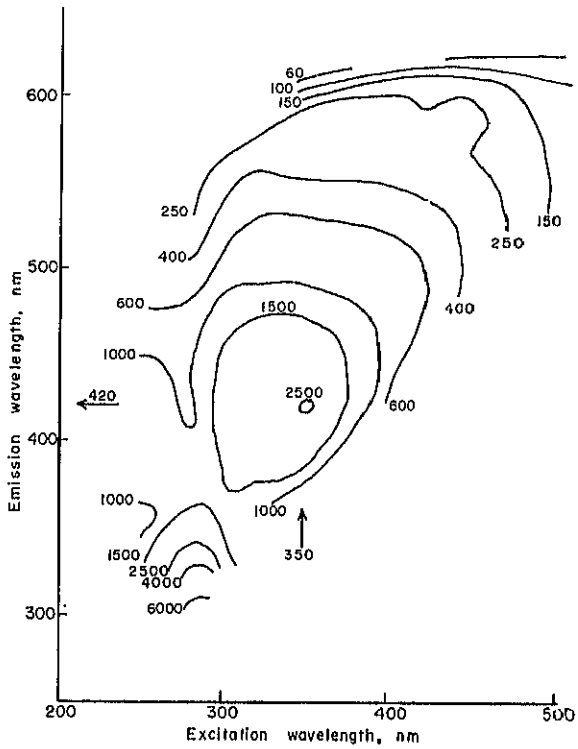


FIGURE 16.—Fluorescence diagram of Australian raw sugar.

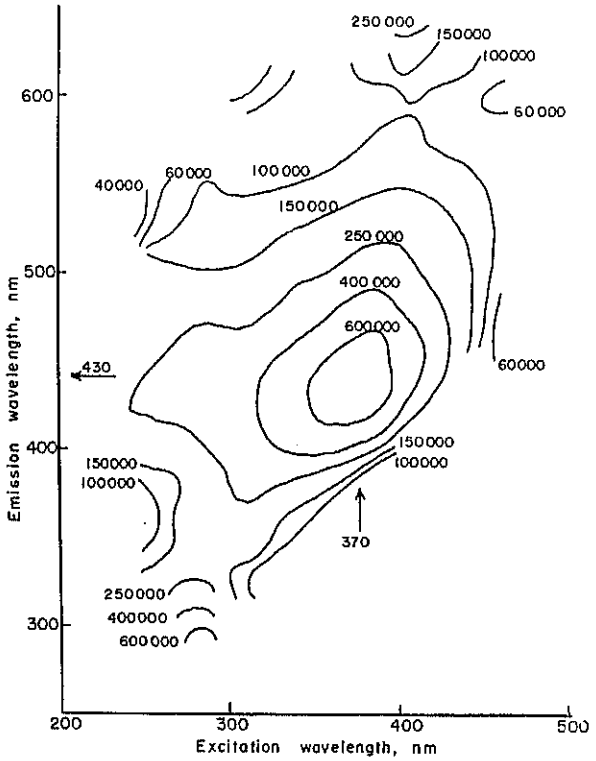


FIGURE 17.—Fluorescence diagram of nondialyzable molasses browning polymer.

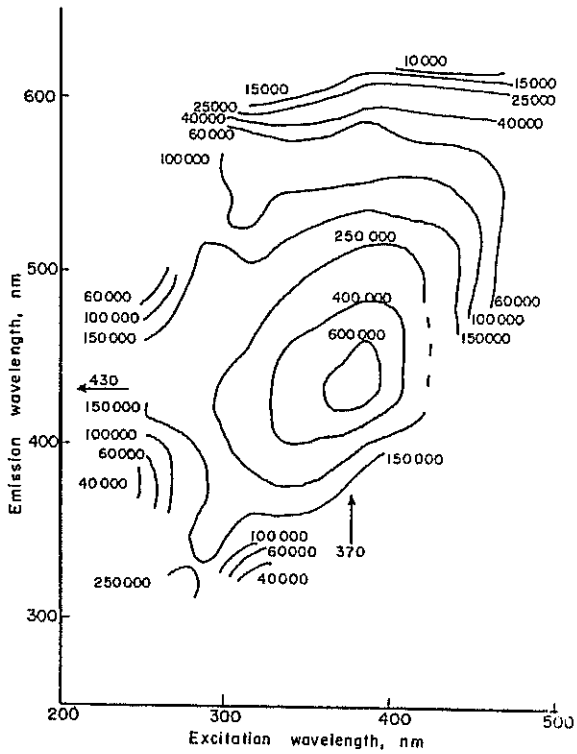


FIGURE 18.—Fluorescence diagram of dialyzed molasses browning polymer.

spicuous absence of the peak at (400,600). This means that this peak is composed only of material of high molecular weight. The other peaks are probably composed of several components of both high and low molecular weight.

The phosphate-clarified refinery liquors show the main peak split into two parts (figs. 19 and 20). One peak is at about (370,430). It may be that the phosphate clarifier is removing material that has its peak midway between these two peaks and so is letting these two show up.

The decolorized liquors in figures 21 and 22 show remarkable differences depending upon the decolorizing agent. Bone char leaves a peak at (300,400) whereas granular carbon leaves a peak at (380,440). The difference is striking and shows beyond a shadow of a doubt that bone char and granular carbon are not equivalent, but that they take out different materials.

The refined sugar shown in figure 23 is again reduced to only one peak. But not all so-called refined sugars are the same. The refined sugar shown in figure 24 has the high-molecular-weight peak at (400,600). However, this was a minimally refined sugar; it could only just be classified as refined.

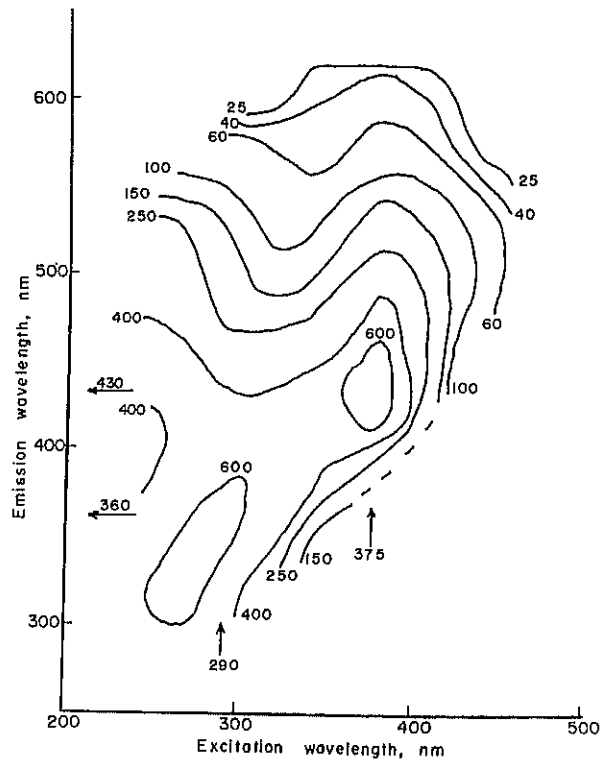


FIGURE 19.—Fluorescence diagram of phosphate-clarified sugar liquor.

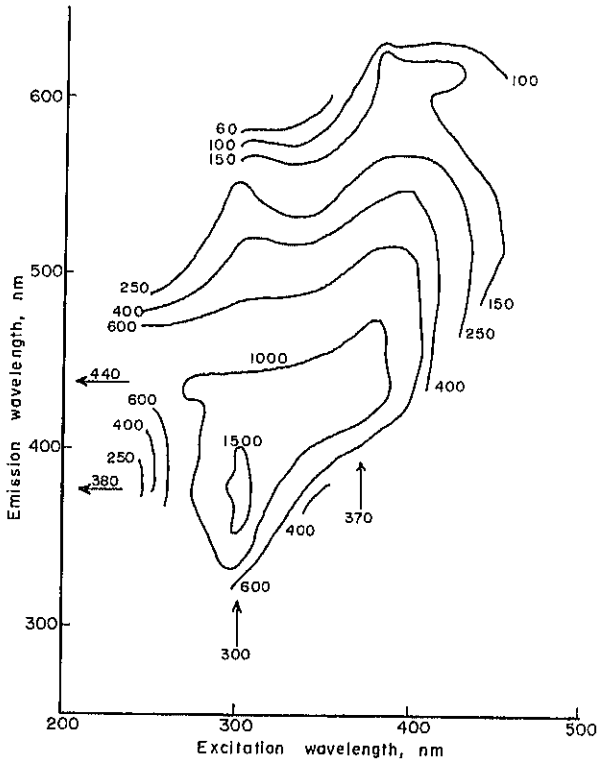


FIGURE 20.—Fluorescence diagram of phosphate-clarified sugar liquor.

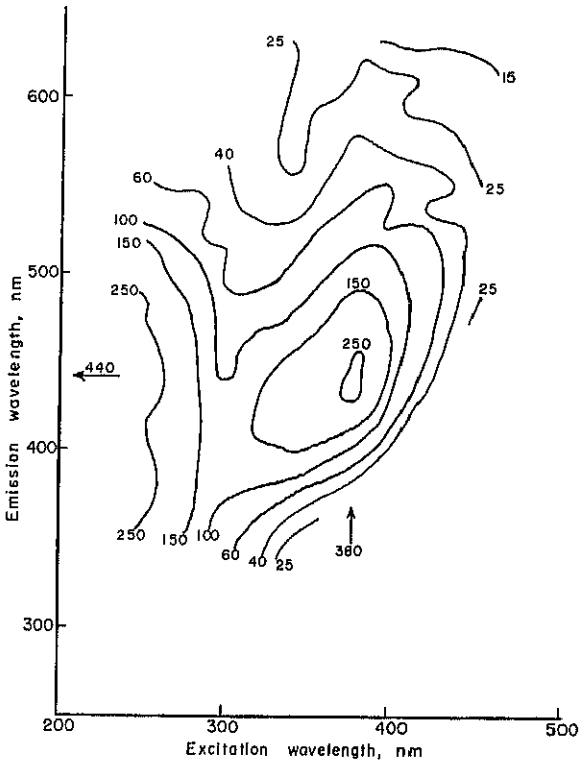


FIGURE 22.—Fluorescence diagram of granular-carbon-filtered liquor.

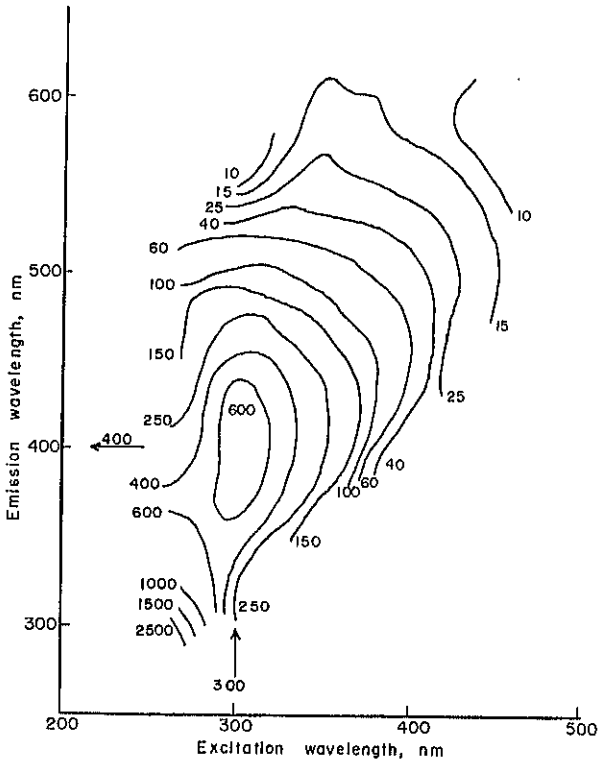


FIGURE 21.—Fluorescence diagram of char-filtered liquor.

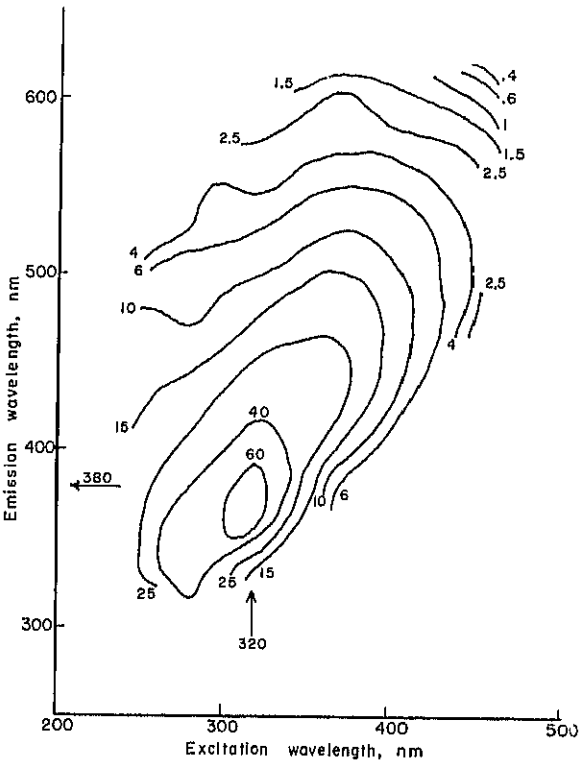


FIGURE 23.—Fluorescence diagram of refined sugar.

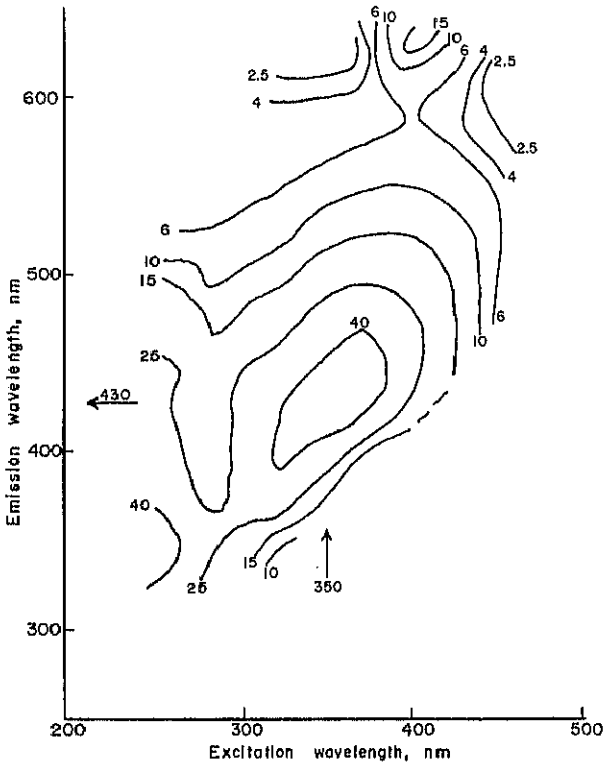


FIGURE 24.—Fluorescence diagram of minimally refined sugar.

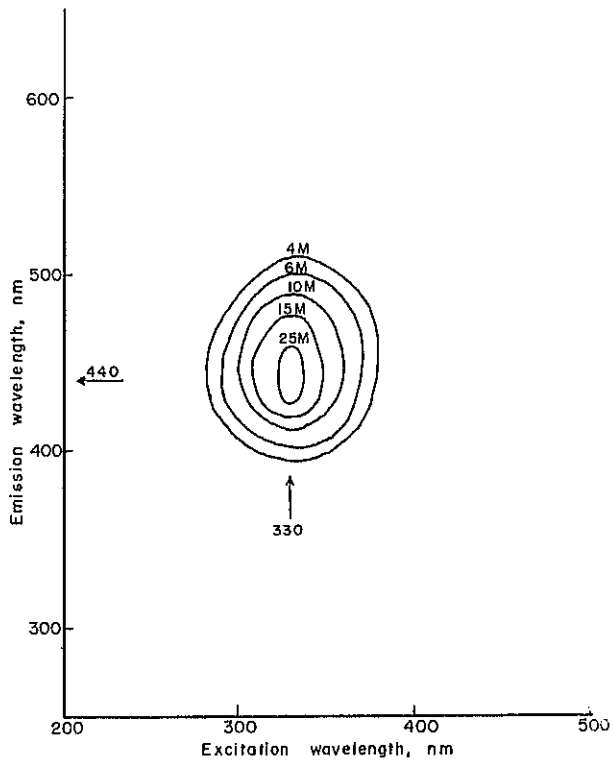


FIGURE 25.—Fluorescence diagram of *p*-coumaric acid.

It is interesting to observe the fluorescence patterns of some known constituents, such as those in figures 25, 26, and 27. Figure 25 shows *p*-coumaric acid. This sharp symmetrical peak is a perfect example of a fluorescence spectrum. If all compounds were so sharply delineated, a mixture could be analyzed with ease. However, the fluorescence patterns are often lopsided, as for ferulic acid (fig. 26), or bimodal, as for sinapic acid (fig. 27). Each of these components has a peak that corresponds fairly well with the usual major peak in sugars and so could be responsible for some of the major fluorescence in sugars.

CONCLUSIONS

In the course of this work it was noted that peaks usually appeared in four places:

1. (360,430).
2. (280,320).
3. (250,430).
4. (400,600).

Peak 1 was the central peak that appeared in all sugars. However, this peak sometimes descended toward peak 2 so that there was really a whole series of peaks between 1 and 2 that could be given fractional numbers. Peak 3 was

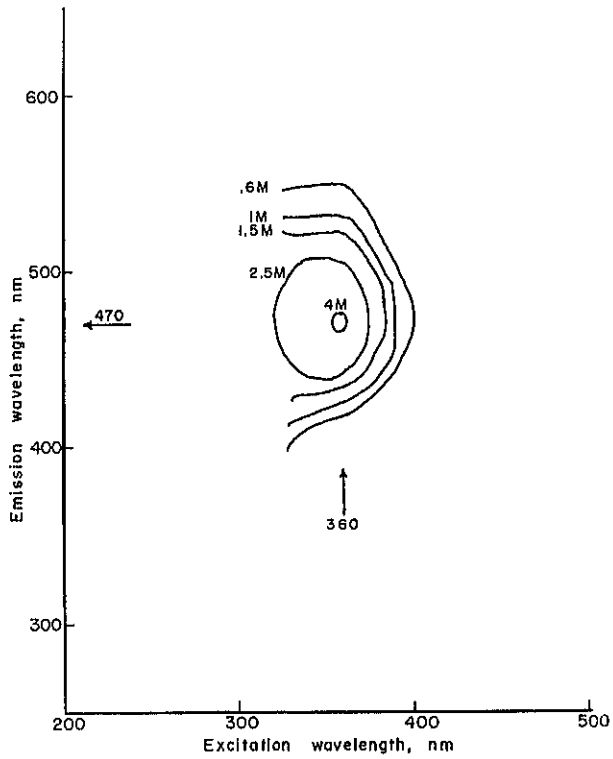


FIGURE 26.—Florescence diagram of ferulic acid.

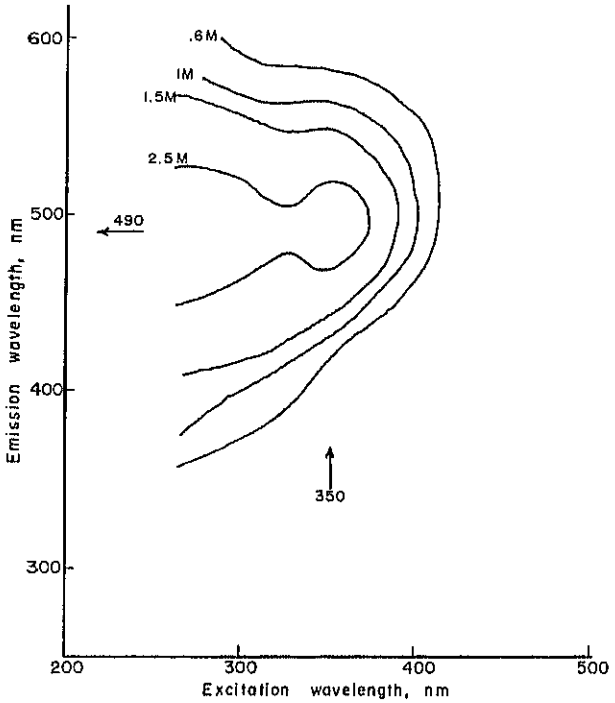


FIGURE 27.—Fluorescence diagram of sinapic acid.

probably just out of the range of the instrument, and the edge of it could be seen. Peak 4 was the high-molecular-weight peak.

The observations are summarized as follows:

	Fluorescent peaks				
Molasses	1			3	
Raws	1		2	3	4
Phosphate-clarified	1		2	3	
Phosphate-clarified		1.5		3	
Char-filtered		1.3	2		
Granular-carbon-filtered	1			3	
Refined		1.5			

In molasses, peaks 1 and 3 overshadowed the others.

In raw sugars, all the peaks were present.

Clarification removed peak 4

Bone char removed peak 3.

Granular carbon removed peak 2.

This is the point at which we find ourselves today in the fluorescence of commercial sugars. The next obvious step is to look at the size of these various fluorescent peaks, where they come from, and where they go in the refining process. We will concurrently continue to try to find exactly what chemical compounds cause these various peaks.

REFERENCES

(1) Argauer, R. J., and White, C. E. 1964. Fluorescent compounds for the calibration of excitation and emission units of spectrofluorometer. Anal. Chem. 36: 368–371.

(2) Demas, J. N., and Crosby, G. A. 1971. The measurement of photoluminescence quantum yields, a review. J. Phys. Chem. 75: 991–1024.

(3) Melhuish, W. H. 1960. A standard fluorescence spectrum for calibrating spectro-fluorphotometers. J. Phys. Chem. 64: 762–764.

(4) Parker, C. A. 1958. Direct recording of fluoresence excitation spectra. Nature 182: 1002–1004.

(5) Parker, C. A. 1962. Spectrofluorometer calibration in the ultraviolet region. Anal. Chem. 34: 502–505.

(6) Parker, C. A., and Barnes, W. J. 1957. Some experiments with spectrofluorimeters and filter flourimeters. Analyst (London) 82: 606–618.

(7) Parker, C. A., and Rees, W. T. 1960. Correction of fluorescence spectra and measurement of flouresence quantum efficiency. Analyst (London) 85: 587–600.

(8) Rieger, C. J., and Carpenter, F. G. 1959. Light scattering by commercial sugar solutions, J. Res. Nat. Bur. Stand. 63: 205–211.

(9) Wall, J. H., and Carpenter, F. G. 1970. Fluorescence of sugars. Proc. 1970 Tech Sess. Cane Sugar Refining Research, pp. 157–178.

(10) White, C. E., Ho, M., and Weimer, E. Q. 1960. Methods for obtaining correction factors for fluorescent spectra as determined with the Aminco-Bowman spectrophotofluormeter. Anal. Chem. 32: 438–40.

DISCUSSION

W. W. BINKLEY (New York Sugar Trade Laboratory): When you started going through those slides, I was sure for a little while that you had a way of fingerprinting raws on the basis of geographical origin, but as your presentation progressed, I was less certain that mine was an accurate conclusion. Now it looks to me as though your findings reflect a combination of geographical origin and of mill performance. Can you comment on that?

F. G. CARPENTER: Although we might hope that we could get such a fingerprint, I do not think that we have that quite yet. I am afraid that this would be oversimplification. I strongly suspect that each peak contains many components, especially peak No. 1. Color measurement had to be reduced to one dimension. At present, fluorescence seems to have four dimensions, and probably it has more than that.

R. MOROZ (SuCrest Corporation): Could you comment on the use of fluorescence as a tool to determine insecticide residues in sugar products, or for determining sugar losses in refineries?

F. G. CARPENTER: As far as insecticides are

concerned, some are fluorescent, and so could be detected this way. We have not actually tried that yet. For sugar losses, fluorescence has possibilities, because when you degrade sugar it becomes fluorescent. We should look at this.

S. B. SMITH (Westvaco): I note there were tremendous differences in the intensity of the fluorescence. Does this bear any direct relationship to concentration? As I recall, from my own work with fluorescence, there are a number of things which tend to quench fluorescence as well as reinforce it, and I wonder how reliable this is as a measure of concentration.

F. G. CARPENTER: Quenching and reinforcing of fluorescence is a further refinement which we have yet to consider. The actual fluorescences as measured were not as different as the corrected values per unit concentration would seem to indicate. This is because the highly fluorescent materials, such as molasses, were diluted to even less than 1 p/m in order to bring them into a similar range on the instrument.

P. POMMEZ (Redpath): I believe that an electronic transition is responsible for the fluorescence, and the value of the wavelength of emission depends on the ionic state of the molecule of interest, and this ionic state depends on the pH. My question is, are you finding any relationship between the pH of solution and the wavelengths of emission?

F. G. CARPENTER: All these studies reported were at a pH of approximately 10 because the pH does indeed make a big difference. Just as pH affects the amount of visible color, so it affects the fluorescence, equally, or even more. If you start juggling pH you get a whole new story, which I did not choose to go into today.

P. POMMEZ: On the samples after bone char and after activated carbon, there was a difference in the peaks in the value of the wavelengths. Did you adjust pH before you measured them?

F. G. CARPENTER: All the pH's were adjusted to be in the vicinity of 10 before we measured them so they are comparable.

G. W. MULLER (Kerr-McGee): You show differences in compounds removed by char and carbon; do you plan to do more work in this area?

F. G. CARPENTER: That, of course, is one of places where we can use fluorescence because here we have another way of measuring types of impurities. Everywhere I go I am asked the same old question, which is better, char or carbon? Everyone is thinking in terms of color removal and I have to explain that color is not one color, but many colorants. With fluorescence we can measure at least four types of them, so this is the way to go to find something new. Yes, we will work in this area.

G. W. MULLER: Can you get any indications about adsorption mechanism from fluorescence?

F. G. CARPENTER: If fluorescence were to show us different types of impurities removed by different adsorbents, then we might get some useful indications about adsorption mechanisms from that data.

N. H. SMITH (California and Hawaiian): The sugars have pigments that fluoresce, which you try to match up to knowns. However, the pigments in the sugar are probably not the simple compounds, but the substituted derivatives with sugars attached. How sensitive is the fluorescence pattern to the attachment of other groups to the simple compound?

F. G. CARPENTER: In general, fluorescence will be sensitive if the electron distribution of the center which is causing the fluorescence is affected by adding a substituent. If the fluorescence center is far removed from the substitution point, then you will not get much difference.

C. J. NOVOTNY (Industrial Filters): Your comparisons of char liquors and those treated by carbon were very interesting. Did you make similar plots for ion-exchange decolorized liquors?

F. G. CARPENTER: No, we did not, but we should do so soon.

C. C. CHOU (Amstar): Referring to the question from Dr. Pommez about the electron transition, I think that the effect of pH would also show up on the phosphorescence. Fluorescence is caused by an electron transfer from a first excited singlet state to ground state, and phosphorescence occurs when the electron falls from a lowest triplet state to ground state. I believe that simultaneous measurements of both phosphorescence and fluorescence spectra would give further insight into the nature of the problem.

F. G. CARPENTER: Some of the fluorescence peaks, some of the fluorescence centers are utterly unaffected by the pH and you find exactly the same fluorescence no matter what pH, but others are very strongly affected by the pH. Phosphorescence might be called a kind of de-

layed fluorescence with an added time factor, but it is also similarly dependent upon pH.

N. H. SMITH: I'm reminded of another problem of pH effects: have you gone into concentration effects either in sugars or in the knowns themselves? At higher concentrations you get a different pattern than you do at low concentrations.

F. G. CARPENTER: It is a common occurrence with fluorescence that it is not linear with concentration over a wide range. You get an inner quenching of flourescence when you get the molecules close enough together, which is in addition to the transmission-correction factor mentioned here. Our solutions were dilute enough so that the concentration effect was not great, but the sucrose might be quenching it.

N. H. SMITH: I was not referring as much to intensity as I was to shifting of the peaks.

F. G. CARPENTER: The shifting of the peaks is sometimes an instrumental problem because the data were not corrected for source and response. One thing that we have done is to take the instrument error out of it. This is data that you could obtain on another instrument. This is an equal-energy fluorescent spectrum.

W. W. BLANKENBACH (Chapman Associates): Is there any particular problem in preparing solutions for this fluorescent reading, bearing in mind the Tyndall effect where you have a haze in the solution. This is a rather similar situation.

F. G. CARPENTER: It can create great difficulties. In general, you want to have at least some transmission throughout the ultraviolet region because we are measuring down to about 250 nm. You cannot have less than about 5% transmission, because if you get less transmission you get no light through the cell as far as the point of measurement. It all gets absorbed in the sample cell before it gets to where you are looking at it. So you have to dilute the sample to the point where you can get some light all the way into the active center of that little sample cell. This means that you must have not below about 5% transmission all the way through the ultraviolet, which means that in the visible region you nearly always have around 90% or better. When the solution gives a lot of scattering, then you get a big scattering peak, but you ignore that in fluorescent measurements. There is also a Raman peak of water, which you do not measure. So there is a little problem in sample preparation, but it can be solved by diluting until the color is just the faintest pale yellow.

PHYSICAL CHEMISTRY OF PHOSPHATATION AND CARBONATATION

By M. C. Bennett[1]

ABSTRACT

Phosphatation and carbonatation were compared with filtration as defecation methods. The removal of turbidity, color, total ions, starch, sulfate, calcium plus magnesium, and total alcohol precipitate was evaluated. It was found that good defecation involves the removal of particulate impurities down to a particle diameter of the order of 0.1 μm. In phosphatation and carbonatation the inorganic precipitate acts as a scavenger system. Phosphatation acts primarily by flocculation, whereas carbonatation acts primarily by the inclusion of impurities within the inorganic crystal. The quantity of precipitate generated in carbonatation is about 30 times that for phosphatation. For this reason, carbonatation shows a substantial removal of those inorganic ash components which form sparingly soluble calcium salts.

Operation of the two processes in practice is dominated by the requirements of the separation treatments. For good floatation in phosphatation, the two major requirements were found to be proper attachment of air bubbles and an adequate floc size. Both requirements may be achieved with chemical additives. For good filtration of carbonatated liquor, the precipitation reaction conditions must favor crystal growth and reduce the possibility of unwanted nucleation in a manner somewhat analogous to sucrose crystallization.

INTRODUCTION

Liquor defecation is essentially a pretreatment for the decolorization stage of sugar refining and ideally, the choice of defecation process should be matched with the requirement of the decolorization adsorbent. In general, the use of a porous granular adsorbent for liquor decolorization should impose a mechanical limitation on the particle size of impurities which escape defecation. There should be no restriction of liquor flow through the adsorbent bed caused by blockage of intergranular spaces. The particle diameter here would probably be in excess of 10 μm.

In no adsorbent, including powdered vegetable carbon and ion-exchange decolorizing resins, should there be restriction of molecular diffusion within the adsorbent particle caused by pore blockage. Knowledge of the pore structure in the adsorbent particle might aid specification of a standard for the defecation treatment, but, unfortunately, only limited data are available. In bone char, for example, it has been calculated (*3*)[2] that most of the available surface lies in pores of 0.02 μm diameter, but this would set a quite unattainable limit for any simple defecation process. Electron micrographs of char particles show the main channels within each granule to be around 0.2 μm in diameter, which is a much more realistic limit for impurity particle size.

The chemical requirements of the adsorbent are even more obscure and in many cases must be overridden by other considerations. For example the pH at which activated carbon surfaces generally show the greatest adsorptive capacity for anionic adsorbate lies well below the minimum value which might be tolerated on the basis of invert formation. Only in the case of bone char and ion-exchange decolorizing resin has there

[1] Research director, Tate & Lyle Technical Services Ltd., Cosmos House, Bromley Common, Bromley BR2 9NA, England.

[2] Italic numbers in parentheses refer to items under "References" at the end of this paper.

been any indication of adsorbent sensitivity to the chemical composition of the liquor. In both cases, the sensitivity arises from the relation between electrical properties of the adsorbent surface and the ionic composition of the liquor (*7, 10*).

It must be concluded that there are few known criteria which can be usefully applied in the assessment of defecated liquor quality. Some attention has, therefore, been given in this paper to the measurable functions of the three most commonly employed defecation treatments: filtration through kieselguhr, phosphatation, and carbonatation. The size limitations mentioned above focus attention sharply on the filtration aspects of each process and an attempt has been made to distinguish between mechanical and chemical removal of impurity.

MATERIALS AND METHODS

Membrane Filters

Millipore membranes of pore size ranging from 8±1.4 μm down to 50±3 nm in 11 steps were used in most work. For the smallest pore diameters, Membranefiltergesellschaft ultra filters of 5 to 10 and Δ5 nm pore size were used. Liquors were filtered at 50 Brix under pressures ranging from 0 to 60 lb/in^2.

Liquor Appearance

Liquor colors were measured in 1-cm cells with the Talameter sugar colorimeter, 50- to 60-Brix liquors were adjusted to pH 7.5, and attenuation was measured at 420 nm. Results are expressed as the attenuation index a^*_{420} at 1 g solids/ml.

Turbidity was measured in 1-cm cells with a Unicam SP 600 spectrophotometer, and 50- to 60-Brix liquors were buffered at pH 3.5 by addition of a little glacial acetic acid (5 drops/10 ml liquor), so that traces of inorganic precipitate (e.g., calcium carbonate or calcium phosphate) could not interfere with the measurement. The attenuation was measured at 900-nm wavelength and results are expressed as the attenuation index a^*_{900} calculated at 1 g solids/ml. The a^*_{900} value was taken to give an indication of turbidity, optical clarity to the naked eye being found at $a^*_{900}=0$.

Constituent Analysis

As an indication of the total content of colloidal material, together with its associated inorganic components, the concentration of material precipitated in 75% ethanol at pH 3.5 (acetic acid buffer) was determined. The precipitate was separated in a centrifuge, washed with 75% ethanol and absolute alcohol, and finally dried under vacuum over P_2O_5. The weight of precipitate is recorded as the total alcohol precipitate (TAP mg/100 g solids). It is believed that this material will include all impurities in the original liquor which are either present as, or capable of entering, the colloidal state.

Starch was determined by a modification of Alexander's method (*1*), and protein by the micro-Kjeldahl technique on the weighed, total alcohol precipitate. Results are expressed as mg/100 g solids. Silica was determined by Alexander's molybdenum blue method (*2*) and is expressed in the same units.

Total anions and cations were determined with the ion-exchange resins IRA 401 and IRC 120, respectively, Cl^- being determined by an EEL chloride meter and H^+ by titration with NaOH. Results are expressed in microequivalents per gram of solids.

Sulfate was determined as $BaSO_4$ turbidimetrically, and phosphate by the molybdenum blue method using 1-amino-2-naphthol-4-sulfonic acid as the reducing agent. Ca^{++} + Mg^{++} was determined by EDTA. Carbonatation and phosphatation techniques are described in the relevant following sections.

RESULTS AND DISCUSSION

Defecation by Filtration

It is widely appreciated that laboratory filtration tests are often empirical in nature and that the results of filtrate analysis depend greatly on the way in which the filtration is carried out. The most serious complications arise through (1) progressive blinding of the filter medium which progressively reduces the porosity of the medium, and (2) the difference between laboratory tests in which the liquor is passed through a fixed bed of filter medium and the industrial practice in which fresh filter aid is added continuously to the filter supply liquor.

Within the limitations of the particular technique adopted, it is reasonable to assume that the known porosity of a clean filter medium sets an upper limit on the particle size of impurities which may pass through it. Provided no attempt is made to give significance to small changes, the

results can at least be used as a basis for comparison with other defecation treatments.

Successive Membrane Filtration

In this series, two types of liquor were used—liquor A of high color and turbidity and liquor B of low color and turbidity. A sample of each of the two washed raw liquors at 50 Brix was passed at room temperature through a set of membrane filters of decreasing porosity, the filtrate from one filtration being passed through the next lower porosity membrane. Care was taken to ensure that filtrate was collected only during the period of effectively constant flow. With the membranes of lowest porosity, the filtration sometimes took over 20 h.

Samples of filtrate were withdrawn at each stage for color and turbidity determination and the results are shown as function of membrane pore diameter in figure 1.

Both liquors show a similar pattern:

1. Turbidity decreases to zero in the range 1–10 μm.

2. Over the range 0.1–10 μm, color is extremely sensitive to the filter porosity. This highlights the inherent dangers in quoting a washed-raw liquor color without reference to details of the sample preparation.

3. Color tends to a plateau value over the range 0.01–1 μm. This presumably indicates the range of filter porosity which should be used for washed-raw liquor-color determinations. Practical considerations require that the filtration should take only a few minutes and a satisfactory compromise may be reached using filters of pore diameter around 0.5 μm.

Filtration Through Filter Cakes

In this series, a more complete analysis was made of filtrates through filter cakes of kieselguhr and a coarse precipitated chalk. The first runnings of filtrate were discarded to minimize the possible effects of adsorption of impurity by the filter cake. The kieselguhr chosen was Dicalite Speedflow with an apparent pore-leakage diameter of 0.5 μm, a grade commonly used for the polish filtration of phosphatation-clarified liquors. The kieselguhr was deposited as a cake comprising 50 mg/cm^2 of filter surface on two Whatman No. 42 papers. The volume of filtrate collected was equivalent to a filter aid use of 0.25% on solids.

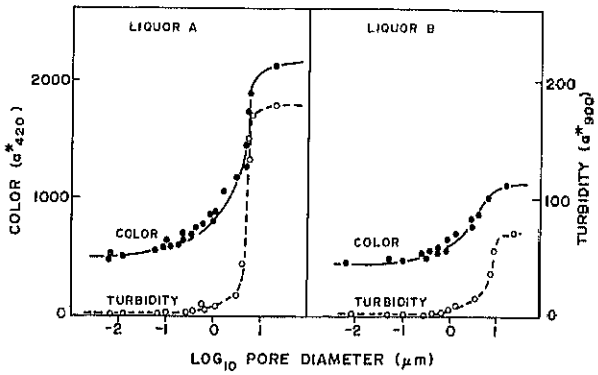


FIGURE 1.—Color and turbidity after membrane filtration of liquors A and B.

The coarse precipitated chalk was Waterworks Chalk Grade 311, which gives liquor filterability characteristics similar to those of normal liquor carbonatation precipitates. It was deposited as a cake comprising 300 mg/cm^2 of filter surface on two Whatman No. 42 papers. The volume of filtrate collected was equivalent to a carbonatation level of 1.0% CaO on solids. Filtrate analyses are given in table 1; the two filter cake results for liquor B are compared with those for the filtrate through a Millipore membrane of pore diameter 0.22 μm.

The results are as expected and show the coarse chalk to have considerably higher leakage porosity than the kieselguhr. Reference to figure 1 shows that the color passing through the chalk cake was equivalent to that passing through a membrane of pore diameter in the range 3–5 μm, whereas the color passing through the kieselguhr cake was equivalent to that passing a membrane of pore diameter in the range 1–2 μm. The removal of "colloidal" impurity from the two liquor types is in accordance with this difference, the kieselguhr giving better defecation than the chalk with respect to TAP, starch, and protein. A further improvement in defecation is achieved when the effective pore diameter is decreased to 0.22 μm by using a membrane filter, and it will be shown later that, with the exception of color, the analysis now approaches that yielded on phosphatation.

The ion balance results reveal the extraordinarily low quantity of ash constituents associated with the suspended or colloidal organic impurity.

TABLE 1.—*Defecation by filtration through chalk and kieselguhr*

Constituent	Liquor A			Liquor B			
	Original	Chalk	Kieselguhr	Original	Chalk	Kieselguhr	Membrane
Color ..... a^*..	2,170	1,420	964	1,120	722	660	600
Turbidity ..... a^*_{900}, pH 3.5..	179	51	12	70	6	5	1
TAP ..... mg/100 g..	200	185	146	151	81	71	67
Starch ..... mg/100 g..	38	31	21	4	4	3	1
Protein ..... mg/100 g..	11	7	5	7	4	3	3
Total anions ..... μeq/g..	19.4	18.4	18.2	16.6	16.4	16.4	15.9
$SO_4^=$ ..... μeq/g..	8.6	8.2	8.7	7.8	7.7	7.8	7.8
$PO_4^{\equiv}$ ..... μeq/g..	2.5	1.2	1.2	0.8	0.5	0.5	0
Cl^- ..... μeq/g..	1.9	1.8	1.8	1.6	1.8	1.6	1.7
$Ca^{++}+Mg^{++}$ ..... μeq/g..	14.2	13.4	12.0	9.8	10.8	9.3	9.3
$Na^+ + K^+$ ..... μeq/g..	5.2	5.0	6.2	6.8	5.6	7.1	6.6

Chalk: Waterworks Grade 311 at 1.0% on solids.
Kieselguhr: Dicalite Speedflow at 0.25% on solids.
Membrane: Millipore 0.22 μm.

Defecation by Chemical Methods

Carbonatation

The sensitivity of impurity removal to filter pore size suggested that, in order to study the chemical effects of carbonatation, it would be necessary to adjust each carbonatated liquor to the same filterability by adding inert filter aid. Data concerning the relative quantities of carbonatation precipitate and Waterworks 311 Chalk required to give constant filterability were already available in the laboratory on liquor C, and it was convenient to make up these mixtures for filtrate analysis. Liquor carbonatation was carried out by the standard constant-condition laboratory procedure, details of which have been published elsewhere (*5*). It has been shown that this technique yields a carbonatation precipitate very similar to that achieved in standard refinery carbonatation practice, and the specific filtration resistance, r, lies between 10^9 and 10^{11} cm/g. The constant conditions were pH 8.0, 75° C, 65 Brix, and 1 h retention time. Waterworks 311 Chalk filter aid was added after carbonatation. The lime addition was varied over the range 0%–1.6% CaO on solids. The changes in filtrate analysis with CaO % are shown in figure 2.

The curves for organic impurities have roughly the same form and most of the defecation is achieved over the range 0%–0.5% CaO. Without the addition of Waterworks Chalk 311 filter aid, optimum filterability is found in liquor C at 1.2% CaO, and it is clear that most of the additional lime required to reach optimum filterability serves only to grow large precipitated $CaCO_3$ conglomerates.

In order to examine further the effect of calcium carbonate precipitation, the above experiment was repeated using a carbonatation technique which produces the smallest precipitated $CaCO_3$ particle size and, hence, yields the lowest filterabilities. The method is the simple batch carbonatation system, whereby the CO_2 is bubbled through a beaker containing prelimed

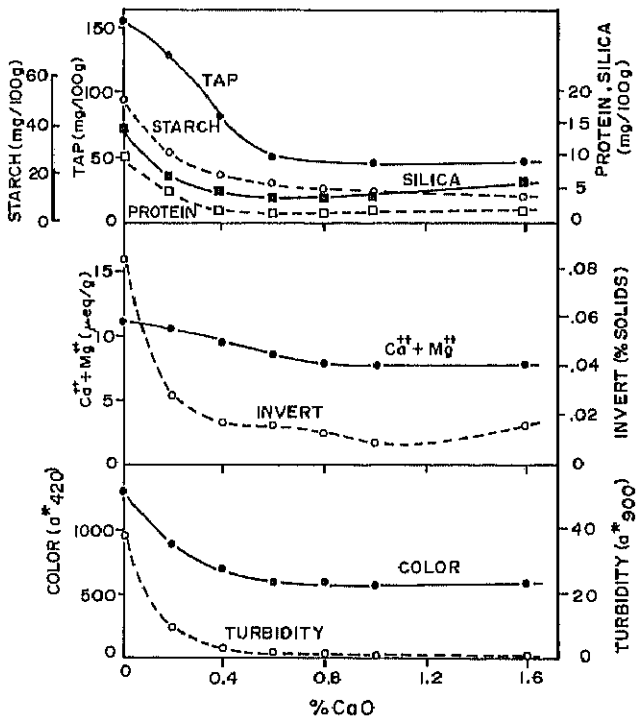


FIGURE 2.—Defecation by carbonatation of liquor C.

liquor at the required level of % CaO on solids. The method has been described and the reasons for the difference in characteristics of the chalk precipitate have been discussed in detail elsewhere (*5*). The nominal conditions for the precipitation reaction were the same as before, that is, final pH 8.0, temperature 75° C, 65 Brix and 1 h total saturation time. The specific filtration resistance of carbonatation precipitates produced in this way can be 100 times greater than that of the normal carbonatation process, and large quantities of filter aid (Waterworks 311 Chalk) are necessary to separate any filtrate.

Batch carbonatations of liquor C were carried out over the range 0%–1.2% CaO on solids, and the filtrates were analyzed as before. Impurity concentration curves were found to be very similar to those shown in figure 2 for the normal carbonatation procedure. Table 2 (last column) lists the analyses for 0.5% CaO. The results are compared with those obtained by the normal carbonatation procedure at the same lime level (taken from figure 2). Analyses are also given for simple filtration of the original liquor through a filter cake of Waterworks 311 Chalk added at the level of 0.5% CaO on solids in the absence of any further carbonatation using lime and CO_2.

The results in table 2 allow a direct comparison of physical and chemical methods of defecation based on calcium carbonate. The chemical methods are outstanding, and there are relatively small differences between the two types of precipitation reaction.

The specific filtration resistance (r) of the batch carbonatation precipitate is of the order of 100 times greater than that of the constant-condition carbonatation precipitate and, hence, according to the Kozeny-Carmen equation, the specific surface might be expected to be 10 times greater. This is an important consideration, because it leads to the conclusion that the extent of defecation in carbonatation is not particularly sensitive to the surface area of precipitated $CaCO_3$. Thus it is unlikely that a simple adsorptive process can be involved; rather, the defecation effect concerns the incorporation of impurity within a growing crystal of $CaCO_3$ by chemical coprecipitation.

In support of this view, crystallographic examination of carbonatation precipitate particles reveals extensive distortion of the normal calcite form, and the X-ray diffraction pattern is displaced from that found with pure calcite. Furthermore, experiments tracing the desorption of a particular impurity, for example $SO_4^=$, show that the total quantity of impurity present within the carbonatation precipitate is only released when the solid phase has been completely dissolved in dilute acid.

In order to examine the possibility that some precipitation of impurity occurs merely on addition of lime, the above experiments were repeated without saturation by CO_2. Samples of liquor C were limed over the range 0%–1.2% CaO, heated for 1 h at 75° C, and then filtered with an addition of Waterworks 311 Chalk.

The filtrate analyses in figure 3 provide no evidence for the precipitation of impurity by lime and therefore support the view that chemical inclusion of impurity within the growing calcium carbonate crystal is the essential mechanism of defecation by carbonatation.

The destruction of invert seen in figures 2 and 3 is a well-known feature of the carbonatation

TABLE 2.—*Defecation of liquor C by* $CaCO_3$ *systems*

Constituent	Original	Filtration through chalk	Carbonatation at 0.5% CaO	
			Continuous	Batch
Color ..... a^*_{420} ..	2,480	1,270	640	620
Turbidity ..... a^*_{000} ..	312	37	1	1
TAP ..... mg/100 g ..	280	156	48	42
Starch ..... mg/100 g ..	78	51	14	10
Protein ..... mg/100 g ..	18	11	2.8	3.1
SiO_2 ..... mg/100 g ..	15	11	6	6
$Ca^{++}+Mg^{++}$ ... μeq/g ..	12.4	11.0	8.6	9.0
$SO_4^=$ ..... μeq/g ..	8.2	8.1	1.5	1.5

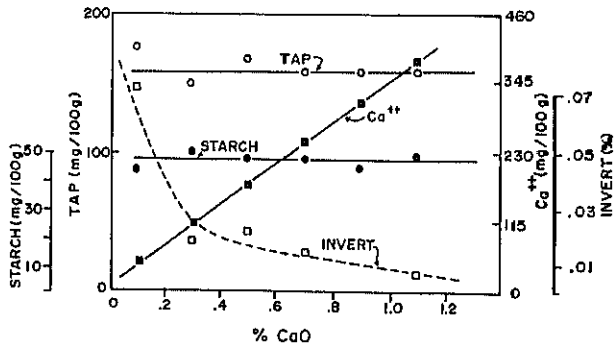


FIGURE 3.—The effect of lime on liquor C.

process and a direct consequence of prolonged contact between liquor and lime at high temperature. The rate of destruction of invert is, of course, quite sensitive to temperature and time of contact.

Phosphatation

Liquor phosphatations were carried out in the following manner: 0.5 *M* phosphoric acid was run into the liquor at room temperature to give the required concentration, and milk of lime was added immediately, raising the pH to the required value. The liquor was heated to 85° C for 50 minutes and then transferred to centrifuge bottles for separation at 1,000 *g* for 90 minutes. The supernatant liquor was decanted and further treated by filtration through a kieselguhr filter cake (Dicalite speedflow, 50 mg/cm²), the volume of filtrate being taken to give an equivalent filter aid use of 0.25% on solids. Both the supernatant and filtrate liquors were analyzed.

A typical phosphatation reaction is described by the results shown in figure 4 for liquor C treated with phosphoric acid equivalent to 0.02% P_2O_5 on solids, limed to the pH values indicated.

The extent of calcium phosphate precipitation is revealed by the filtrate content of phosphate and the greatest change occurs around pH 6. The filtrate color (measured on liquor samples adjusted always to pH 7.5) follows the precipitation around pH 6, but there is further color removal at high pH values. The extent of flocculation is revealed by the turbidity of the supernatant liquor after centrifugal separation (the lower the turbidity of the supernatant liquor, the higher the degree of flocculation in the original, treated liquor). However, the flocculation reaction lags slightly behind the precipitation reaction. The result is similar to that found in the carbonatation reaction: the first precipitate to appear in the liquor apparently deals primarily with impurity removal, while the later additions of precipitate deal with aggregation of the solid phase already present.

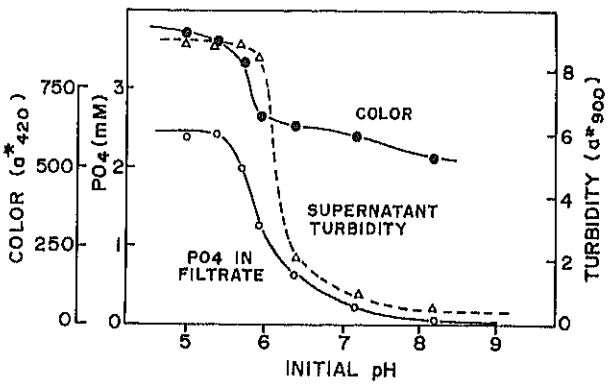


FIGURE 4.—Phosphatation of liquor C.

Comparison of Phosphatation and Carbonatation

Carbonatations were carried out by the laboratory constant-condition method, which gives the same filterabilities achieved in refinery plants. Phosphatations were carried out as described above, liming to pH 8.0.

The analyses of filtrates from liquor A, carbonatated over the range 0%–0.9% on CaO and phosphatated over the range 0%–0.04% P_2O_5 are shown in figure 5. Although no added filter aid was used in any carbonatated liquor, the values for zero lime are taken from the chalk filtrate analyses in table 1. All phosphatated liquors were polish-filtered through a filter cake of Dicalite Speedflow kieselguhr used at the rate of 0.25% on solids, and the values for zero phosphate addition are taken from the kieselguhr filtrate analyses shown in table 1. The results shown in figure 5 are presented using the same set of axes for both phosphatation and carbona-

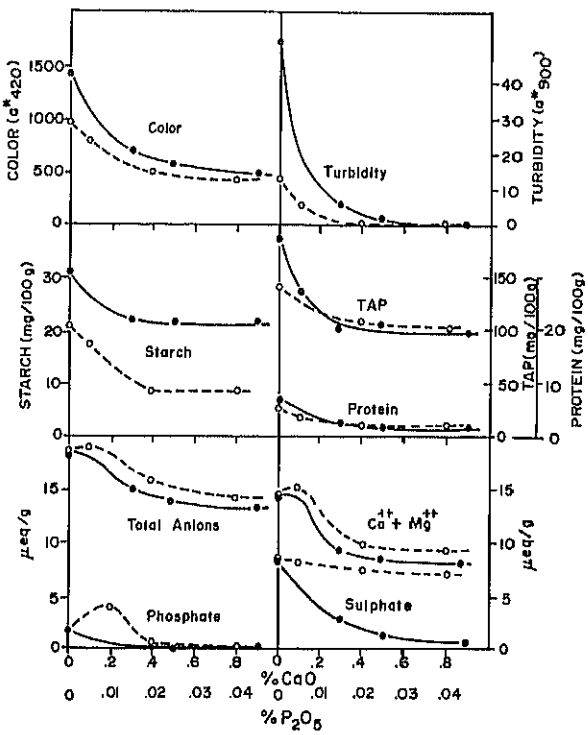


FIGURE 5.—Comparison of defecation by carbonatation (solid lines) and phosphatation (broken lines) in liquor A.

tation. The scale has been chosen so that 0.03 % P_2O_5, approximately the normal level for plant-scale phosphatation, is equivalent to 0.6 % CaO, approximately the normal level for refinery plant carbonatation.

The important features of the analyses shown in figure 5 may be listed as follows:

1. In starch removal, phosphatation is much more effective than carbonatation.

2. In $Ca^{++}+Mg^{++}$ and sulfate removal, the reverse is true.

3. The removal of TAP by the two processes in this liquor A is seen to be similar, but this is not always the case. Generally, carbonatation is found to be considerably more effective than phosphatation.

4. In the removal of color and turbidity, phosphatation shows a slight advantage, particularly at the lower defecant levels. This will be discussed in greater detail later.

5. The gain in $Ca^{++}+Mg^{++}$ and phosphate content at very low % P_2O_5 levels is probably due to the presence of very finely divided calcium phosphate which escapes both the centrifugal separation and the polish filtration.

The results for liquor B shown in figure 6 are consistent with those for liquor A in figure 5, though the level of organic impurity is much lower. The difference between carbonatation and phosphatation is evident in the curves for starch, TAP, $Ca^{++}+Mg^{++}$, and sulfate. The gain in $Ca^{++}+Mg^{++}$ and total anion content of the phosphatated liquor filtrates at low % P_2O_5 levels can probably be explained, as before, by leakage of calcium phosphate precipitate through the kieselguhr filtration. Color is removed to an almost equal extent in both processes.

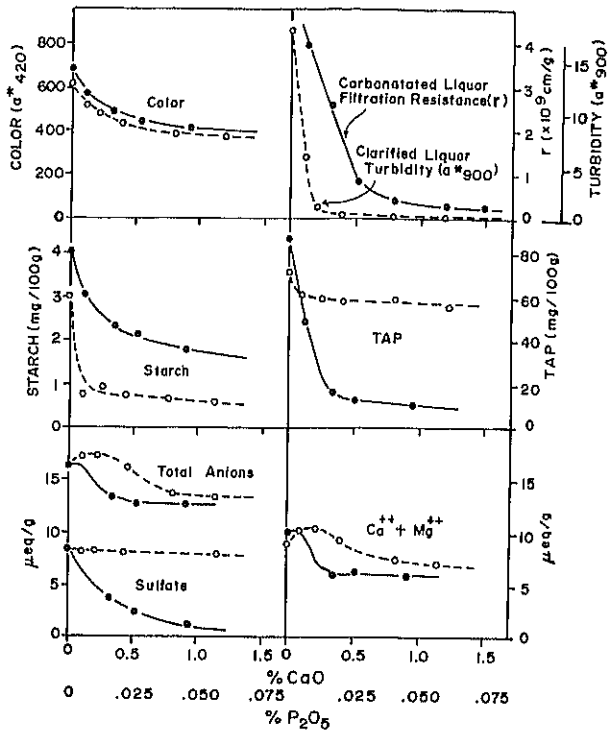


FIGURE 6.—Comparison of defecation by carbonatation (solid lines) and phosphatation (broken lines) in liquor B.

For the sake of completeness, figure 6 (top right) also shows the carbonatated liquor specific filtration resistance (r) and the phosphatated liquor turbidity before polish filtration. These are the factors which affect operation of the two processes in practice and which will be discussed in a later section.

In table 3 the findings on liquors A and B have been summarized in a comparison of the results of carbonatation at 0.6 % CaO with those of phosphatation at 0.03 % P_2O_5. The original an-

TABLE 3.—*Comparison of carbonatation at 0.6 % CaO with phosphatation at 0.03 % P_2O_5 in liquors A and B*

Constituent	Liquor A			Liquor B			
	Original	Carb.	Phos.	Original	Carb.	Phos.	Membrane
Color ..... a^*_{420}..	2,170	560	480	1,120	440	420	600
Turbidity ..... a^*_{900}..	179	1	0	70	1	0	1
TAP ..... mg/100 g..	200	98	105	151	14	58	67
Starch ..... mg/100 g..	38	22	8	4	2.0	0.7	1.2
Protein ..... mg/100 g..	11	2	2	7	2	2	3
Total anions ..... μeq/g..	19.4	13.6	14.6	16.6	13.0	14.4	15.9
SO_4= ..... μeq/g..	8.6	1.2	6.2	7.8	1.5	7.4	7.6
PO_4≡ ..... μeq/g..	2.5	0	0.3	0.8	0	0	0.4
$Ca^{++}+Mg^{++}$ ..... μeq/g..	14.2	6.8	9.3	9.8	6.0	8.6	9.2

alysis of (unfiltered) washed raw liquor is given in each case.

The important features of this comparison are as follows:

1. The percentage color removal shown by phosphatation and carbonatation are similar and in the range of 70 %–80 %. These figures are considerably higher than those generally quoted for the defecation process because they are based on the colors of unfiltered, washed raw liquor. Generally, washed raw color is determined after filtration through kieselguhr or Millipore membranes and the color recorded is, of course, much lower. On this basis phosphatation and carbonatation are generally found to achieve decolorization levels below 50 %.

2. At the chosen defecant levels, the phosphatation liquor colors are slightly better than the carbonatation liquor colors, in contrast to what is often found in practice. From a survey of refining operations (*9*), it has been reported that phosphatation refineries obtain 25 %–40 % decolorization, whereas carbonatation refineries obtain 30 %–50 % decolorization. The data presented in this report were obtained under the more tightly controlled laboratory conditions and might be taken to indicate that, although phosphatation is capable of yielding a small color advantage, this is seldom achieved in refinery practice. It should also be noted that the phosphatation reaction pH used here (pH 8.0) is probably higher than that commonly used in refinery practice, and gives slightly superior decolorization results, as shown in figure 4.

3. Both processes achieve almost complete removal of turbidity, and this must be highlighted as one of the major features of the chemical defecation treatments. In fact, phosphatation at 0.03 % P_2O_5 was found to achieve 100 % removal, whereas carbonatation at 0.6 % CaO achieved a slightly lower removal. Again, it is doubtful that the slight advantage offered by phosphatation can be realized in practice by the conventional phosphatation processes.

4. Whereas the phosphatation results show a very considerable advantage over carbonatation in starch removal, the TAP results (which include all "colloidal" material) show the reverse effect.

5. As is already well known, carbonatation shows a spectacular removal of sulfate, Ca^{++}+ Mg^{++}, and phosphate. It is believed that this effect of carbonatation concerns the removal of any anion which forms a sparingly soluble calcium salt. Calcium sulfate and calcium phosphate are removed by inclusion within the precipitated chalk particles, and there seems no reason to doubt that the calcium salts of certain organic acids, including acidic polysaccharides and some color, behave in the same way. Approximately 30 times more calcium ion is precipitated in carbonatation than in phosphatation and the possibility of inorganic ash inclusion by this mechanism in phosphatation can, therefore, be neglected.

6. The last column of data for liquor B shows an analysis of the filtrate through a 0.22-μm pore diameter Millipore membrane. The results, taken from table 1, are similar to those of the phosphatation treatment. This comparison might indicate that the defecation action of phosphatation is primarily an aggregation of impurity particles (generally termed "flocculation") and that the maximum capability of phosphatation with respect to most impurities could be defined by filtration through a pore diameter of approximately 0.1 μm. The removal of color would apparently lie outside this broad generalization and it is likely that, in this case, some specific adsorption occurs as with the hydroxyapatite component of bone charcoal.

Separation of Impurities in Carbonatation and Phosphatation

Operation of the carbonatation and phosphatation processes in refinery practice is dominated by the requirements of the separation treatments, filtration in the case of carbonatation, and flotation followed by filtration in the case of phosphatation.

Carbonatation

It is already been shown that the defecation achieved by carbonatation is not particularly sensitive to the way in which the carbonatation reaction is carried out. It is considerably more dependent upon the quantity of chalk precipitated in the liquor. The defecation stage consists primarily of impurity inclusion within precipitated chalk particles; therefore, the ease of removal of this impurity from refinery process liquor relies critcally upon the filterability of the precipitated chalk.

Several of the facters which affect carbonatated liquor filterability have been discussed in-

dividually in previous publications (*4–6*). For maximum filterability in a given liquor the following factors are important:

1. Percentage CaO. For every liquor there is a particular lime dose at which the filterability will be greatest. This optimum lime dose varies from below 0.4% CaO on solids in some liquors to over 1.2% CaO in others.

2. Retention time. A few carbonatation plants operate with a single continuous-flow saturator; most plants operate with two saturators in sequence; and some have three saturators in sequence. Retention time is vitally important only in the first saturator of the sequence, where most of the $CaCO_3$ precipitation occurs. For maximum filterability, retention time in this first stage of carbonatation should be not less than 45 minutes.

3. pH. For maximum filterability, the pH of the first saturator should be as low as possible and never above pH 10. Ideally, the whole reaction should be carried out at the final pH required, for example, pH 8.0–8.5, in a single saturator. However, gas absorption efficiencies are found to be uneconomically low and a compromise must be found. In a two-saturator system, maximum filterability is generally achieved with a first-saturator pH around 9.5. Filterability is only slightly sensitive to the pH at which the second saturator is operated, the latter serving mainly to adjust the pH to suit later process requirements and to achieve minimum calcium ash content.

The relation between pH and calcium ash content in a particular liquor is, of course, determined by the bicarbonate-carbonate equilibrium conditions for that liquor. In practice it is seldom possible to achieve carbonatated liquor pH values below 8.0 and most processes run around pH 8.2–8.5.

4. Temperature. Temperature affects not only viscosity, with its consequential effect on observed filtration rate, but also the quality of precipitated $CaCO_3$ particles. The degree of conglomeration increases with temperature up to about 82° C, and filterability increases independently of the improvement observed in filtration rates due to reduced viscosity.

Two other variables must be mentioned, Brix and lime quality. Like temperature, Brix affects both filtrate viscosity and particle structure in the $CaCO_3$ precipitate, a reduction in Brix giving an increase in filterability independent of the reduced viscosity. The effects of temperature and Brix on viscosity have an important bearing on the mass transfer rate of CO_2 from the gas phase into solution and thence into the ionic carbonate forms in which it can enter the carbonatation reaction. These two variables must also be expected to have some influence on the nucleation and crystallization rate of calcium carbonate.

Lime quality is a factor appreciated by most carbonatation technologists, but understood by only a few physical chemists. If the amount of available $Ca(OH)_2$ is kept constant in a comparative experiment, slaked lime from limestone is generally found to give significantly higher filterabilities than slaked lime from chalk. Freshly slaked quicklime is considerably inferior to the same milk of lime which has been allowed to stand for a few hours.

The most striking effect is produced when the components of slaked lime, Ca^{++} and OH^-, are added (in the laboratory) from independent sources such as $CaCl_2$ and KOH. The measured carbonatated liquor filterabilities can be up to 10 times greater than those found using conventional milk of lime, as described elsewhere (*6*). The effect is little understood, but there is some evidence that it concerns the nucleation of calcium carbonate by solid phase particles of $Ca(OH)_2$. When the undissolved solid phase $Ca(OH)_2$ is eliminated by the separate addition of $CaCl_2$ and KOH, or removed from a conventional lime sucrate solution by membrane filtration, unwanted nucleation is reduced with a concomitant increase in the size of the carbonatation precipitate particles.

The carbonatation precipitation reaction is essentially a crystallization process, like sucrose crystallization in pan boiling, with the important difference that the level of supersaturation is maintained by chemical reaction between the two added reactants, calcium and carbonate ions. Excessive nucleation must be avoided in both the saturator and the vacuum pan, and similar principles must be followed. Sudden changes in liquor flow rate, reactant flow rate, temperature, Brix, and pH produce an immediate decrease in filterability and in most cases the change can be detected by particle size analysis of the carbonatation precipitate.

An important difference between sucrose crystallization and carbonatation concerns conglomeration of the product crystal. In nearly

every liquor studied in these laboratories, acceptable carbonatated liquor filterability depends upon the establishment of the highest possible degree of conglomeration of precipitated $CaCO_3$ crystallites. Certain impurities which occur in particular liquors have the effect of preventing conglomeration and low filterabilities result. These impurities are effective in low concentration and have not been identified in the type of gross analysis reported earlier in this paper.

When the factors discussed here are taken into account in both the design and operation of a refinery carbonatation station, the process offers an exceptional degree of reliability. The use of a lime dose considerably in excess of that required to achieve defecation ensures relative insensitivity to minor variations in either liquor quality or operating conditions.

Phosphatation

The particle of the phosphatation precipitate is extremely small, and the specific filtration resistance of the phosphatation floc is about 1,000 times greater than that of carbonatation precipitates. A relatively large proportion of high-quality filter aid must, therefore, be added to achieve acceptable liquor filtration rates. In order to reduce the quantity of phosphate floc presented to the filters, a flotation clarification is generally carried out prior to filtration. In the conventional flotation clarifier, the phosphate floc removal is found in practice to lie between 50 % and 95 %, the remaining floc being carried over in the so-called clarified liquor to the filter station.

When the flotation clarifiers are operated with high efficiency, polish filtration of the clarified liquor seldom presents any difficulty and is sometimes omitted altogether. With low-efficiency flotation clarification, the load on the filter station is reflected by the relatively large amount of filter aid required. In this case, the phosphoric acid addition is sometimes reduced to such levels that large part of the defecation effect is lost.

At the normal phosphoric acid dose of 0.02 %–0.03 % P_2O_5, the composition of carryover floc is identical to that of the floated scum material, and its presence in clarified liquor is merely an expression of inadequate flocculation or incomplete flotation within the retention time available. Since the removal of such material by filtration must involve some compromise between the porosity of the filter aid and an acceptable liquor filtration rate, a part of the carryover material will generally pass through the filters. It is this aspect of the conventional phosphatation process which probably accounts for the observations in refinery practice that phosphatation gives somewhat lower color removal than carbonatation.

The technology of the phosphatation flotation process has been the subject of an excellent review (*8*). From the physicochemical point of view, four features of the conventional phosphatation process warrant comparison and contrast with carbonatation.

First, an adequate phosphoric acid dose is necessary to induce flocculation of suspended impurity. From data presented above, this is generally around 0.03 % P_2O_5 on solids, and additions above this level serve only to increase the total quantity of floc.

Second, there is little evidence to suggest that the separation of the phosphate floc is sensitive to the way in which the lime and phosphoric acid reactants are mixed. Because phosphatation is essentially a flocculation process, the smaller the calcium phosphate crystallite, the greater is the defecation effect. Rapid and complete mixing of the two reactants is, therefore, a desirable feature of the phosphatation process and it is common practice to use in-line power mixers where the retention time is less than 1 second. However rapid the initial precipitation, the reaction mixture takes an appreciable time to reach chemical equilibrium. A decrease in pH can be measured several minutes after initial mixing of reactants, and the residual phosphate content also decreases during this period.

Third, because of the simplicity of mixing the two reagents, phosphatation is often used in batch operations. In this case, careful control of pH is essential, since minor fluctuations from batch to batch can lead to further reaction at the mixing boundary between two consecutive batches. In continuous flow operations, the importance of steady-state conditions in the liquor flow, reactant feed rate, and pH of the reaction mixture has been recognized for many years.

Fourth, when clarifiers are used for the primary separation, adequate aeration is essential for satisfactory flotation of the floc. In the conventional phosphatation process, air bubbles are trapped mechanically in the phosphate floc, and a variety of systems have been developed for this

purpose. In most cases it is essential to heat the liquor in the clarifier so that conditions of minimum air solubility in liquor and continued bubble growth are maintained during the period of flotation. Clarifier temperatures as high as 97° C are commonly found in practice.

With the notable exception of one or two refineries, conventional phosphatation flotation is found in practice to be a sensitive process. Relatively minor changes in reaction conditions or in the liquor type can have a catastrophic effect on flotation characteristics, leading to the appearance of heavy carryover in the clarified liquor. In nearly every case the change can be attributed either to a decrease in the level of air bubble retention within the floc or to a decrease in floc size.

During the last 2 years, work in these laboratories has led to the development of chemicals which have a profound influence on the aeration and flocculation of phosphatation precipitates. First, the use of Talofloc as a color precipitant leads to the inclusion of the surface-active dialkyl quaternary molecule within the phosphate floc. This material reduces interfacial tension at the air/liquor interface, so that in the presence of air bubbles, the hydrocarbon chains are orientated at the bubble surface. In effect, in the presence of Talofloc, air bubbles adhere to the floc so that special devices or systems are no longer required to achieve proper aeration. Of course, the air must be presented in a state of subdivision in which a close approach is possible, and this requires a bubble diameter in the range of 10–100 μm. This can be readily achieved by passing the liquor through a centrifugal pump fitted with a cruciform impeller operating at speeds around 3,000 r/min, with an air bleed on the suction side. In the presence of Talofloc, aeration of the floc is spontaneous.

The second chemical, Taloflote, is a flotation aid which influences floc size and controls flotation rate. Investigation of the relationship between molecular structure and activity in acrylic acid-acrylamide copolymers led to its development. Over the dose range of 1–10 p/m Taloflote on solids, the increase in floc size is followed by an increase in flotation rate and a decrease in carryover.

It will be seen that the two specialty chemicals mentioned here have quite different and unrelated effects on the phosphatation floc. They impart physicochemical properties to the floc which can never be matched by those arising naturally during the course of normal processing. In this way, the use of the specialty chemicals overrides many of the inherent properties of phosphatation floc and eliminates the more extreme sensitivity of the system.

CONCLUSIONS

"Turbidity" is the only component of washed raw liquor which can be completely removed by the chemical defecation processes. Since turbidity can also be completely removed by membrane filters of pore diameter approximately 0.1 μm, it is inferred that the appearance of turbidity in liquors is due to the presence of a suspension of particles of diameter greater than approximately 0.1 μm. Chemical analyses show that the composition of such particles covers a wide range of organic and inorganic material and includes the so-called "colloidal" impurities like starch, gums, and protein.

Phosphatation acts primarily by flocculation of impurity particles and, with the exception of color, the maximum possible removal of impurity by this process can be determined by filtration through a membrane of pore diameter 0.1 μm. Color removal is better than that indicated by such a membrane filtration, presumably because of adsorption onto the precipitated calcium phosphate.

Carbonatation acts primarily by impurity inclusion within relatively large masses of precipitated calcium carbonate conglomerates. Approximately 30 times more lime is precipitated as carbonate during carbonatation than as phosphate during phosphatation. Carbonatation therefore shows a remarkable specificity in the removal of any impurity capable of forming a sparingly soluble calcium salt. This accounts for the outstanding feature of the process in removing sulfate and phosphate, together with certain organic and anionic material which may include some color and acidic polysaccharides. For these impurities which are not capable of taking part in such a specific chemical interaction, starch, for example, carbonatation shows a lower removal than phosphatation.

The separation of impurity from phosphatation liquor in refinery practice depends ultimately upon the porosity of the filter aid employed. When a preliminary clarification is carried out

by flotation, the separation depends on satisfactory aeration and flocculation of the phosphate precipitate. In the conventional process this is highly sensitive to minor variations in both the liquor and the process conditions. However, newly developed chemical additives are capable of overriding the natural variations and can eliminate the sensitivity of the flotation system.

The ease of separation of impurity from carbonatated liquors in refinery practice is determined by the filterability of the precipitated $CaCO_3$. Provided the design and operating conditions of the saturators follow the well-known principles of crystallization processes, the carbonatation system is relatively insensitive to minor variations.

ACKNOWLEDGMENTS

The experimental work performed by Mrs. F. J. Gardiner and S. D. Gardiner is gratefully acknowledged.

REFERENCES

(1) Alexander, J. B. 1954. Starch in the sugar industry. Proc. 28th Annu. Congr. S. Afr. Sugar Technol. Assoc., pp. 100–104.

(2) ———. 1957. Some observations on the filterability of Natal raw sugars. Proc. 31st Annu. Congr. S. Afr. Sugar Technol. Assoc., pp. 68–75.

(3) Barrett, E. P., Joyner, L. G., and Halenda, P. P. 1952. Deterioration of bone char and Synthad with use. Ind. Eng. Chem. 44: 1827–1833.

(4) Bennett, M. C. 1967. Liquor carbonatation. 1. Impurity effects on filterability. Int. Sugar J. 69: 101–104.

(5) ———, and Gardiner, S. D. 1967. Liquor carbonatation. 2. Flocculation of $CaCO_3$. Int. Sugar J. 69: 198–202.

(6) ———, and Gardiner, S. D. 1968. Liquor carbonatation. 3. Laboratory procedures for comparing the quality of liquor or lime samples. Int. Sugar J. 70: 135–137, 173–176.

(7) Carpenter, F. G., Larry, D., and Deitz, V. R. 1961. Ionic interactions with sugar colorant during char filtration. Proc. 7th Tech. Sess. Bone Char. pp. 254–291.

(8) Saranin,A. P. 1972. Technology of phosflotation of sugar melt. Sugar Technol. Rev. 2: 1–72.

(9) Symposium on Auxiliary Decolorants and De-ashing Techniques. 1969. Sugar Ind. Technol. 28: 219–223.

(10) Tate & Lyle, Ltd., Technical Services Group research and development. 1970. Annual Report, pp. 5–9.

DISCUSSION

K. R. HANSON (Amstar): That was a great paper on chemistry, but if you wanted to build a plant, what process would you use?

M. C. BENNETT: The choice between carbonatation and phosphatation cannot be made simply on technical consideration of the two defecation processes. Many other background data are necessary. First, the choice depends on economics, where the important factors are operating cost and capital investment. Your financial advisers would have to specify the constraints on the availability of capital and the payback period required for any investment. These factors are important because carbonatation requires a comparatively higher capital investment but has a lower operating cost. In short, it is high on fixed costs but low on variable costs. The Talofloc phosphatation system, with its low retention time, very simple clarifier, high filterability and partial decolorization, requires a comparatively low capital investment but has rather higher operating costs. Another factor concerns the type of decolorization system to be used. As I said in my presentation, ideally the defecation process should be matched with the decolorization process. I suspect, without any real evidence to back my suspicion, that carbonatation goes with bone char and phosphatation goes with granular carbon. Talofloc often produces a better effect with granular carbon than with bone char and certainly goes with ion-exchange decolorizing resins. If a refiner were concerned about bone char supplies in 30 years' time, he might favor phosphatation. A third factor which must be taken into account these days is the requirement of the Environmental Protection Agency regarding disposal of defecation mud. Carbonatation produces considerably more solid waste than does phosphatation. In summary, then, you must consider many points, and will perhaps find 60% in favor of one type of defecation system and 40% in favor of the other.

C. C. CHOU (Amstar): Are the color units shown in table 1 corrected for turbidity?

M. C. BENNETT: No, they are attenuation index units, merely the attenuation of a light beam at 420 nm. Turbidity was measured quite independently by attenuation at 900 nm.

C. C. CHOU: That could be the reason you have such a high percentage of color removal.

M. C. BENNETT: That's right.

C. C. CHOU: Would you care to comment on the rate of color removal by phosphatation? By that I mean the percentage of color removal versus time after phosphatation. Do you have experimental data to show the percentage of color removal versus time?

M. C. BENNETT: No, we don't.

F. G. CARPENTER (Agricultural Research Service): I would like to reinforce your comment about starch in the calcium phosphate. One wonders, how could the starch survive all that time? Some years ago we reported at one of these meetings that we found starch granules in phosphate floc. The starch had apparently been through all the raw house operation, had been boiled to sugar, had gone through the melter and the affination, and had gotten into the phosphate clarifiers, where again it was brought up to boiling temperature. We found that granules were still there. So there is something unusual about cane starch.

H. G. GERSTNER (Colonial): I assume that the phosphatation experiments were run conventionally and not with the use of Talofloc or Taloflote. If you did use those chemicals, would not these results be quite different and in favor of phosphatation?

M. C. BENNETT: They would be different, yes, and very much in favor of phosphatation. In fact, however, most of the data I've shown today were obtained before we discovered anything about these chemicals.

H. G. GERSTNER: Thank you. Another question: Did you ever run this experiment in futility—to try phosphatation followed by carbonatation or vice versa? That is, did you ever take phosphated liquor and subject it to carbonatation to see just what would happen to the color?

M. C. BENNETT: Yes, we did try a mixed process, but we could never get any advantages from it. Mixing the two processes together cancels one out against the other, and you are left with a system you cannot filter or float. Theoretically, if you put them together you should get the best of both worlds, but in our opinion it would never pay for itself.

J. BURT (Amstar): Why does the filterability of carbonatated liquor pass through a maximum at one particular lime dosage? Another question: Earlier in your paper, you mentioned a constant-condition lab procedure that yields a carbonatation precipitate similar to refinery practice. Could you briefly describe that?

M. C. BENNETT: To answer the first question, we must first define the terms used. The average specific resistance, r, is analogous to electrical specific resistance and is a property of the calcium carbonate precipitate. If the quality of this precipitate does not change with increase in the amount of lime used for carbonatation, the specific resistance will be the same at all lime doses. On the other hand, the filterability term, F, is a property of the system as a whole and depends on both r and C, the concentration of chalk precipitate present. F is defined as $1/rC$,[1] and if r remains constant, it is clear that the filterability must decrease with increase in lime dose. However, r is not constant at all lime doses, and, in fact, decreases very rapidly over the range 0%–0.3% CaO. Furthermore, over this range, r decreases more rapidly than C increases, so that there is a net gain in F. As the lime dose is increased still further, r eventually falls to a more or less constant value and at this point F reaches its maximum value. Further additions of lime serve only to increase the total thickness of filter cake, and so F decreases in proportion to the increase in C. Every liquor is characterised by one particular lime dose, at which the carbonatated liquor filterability is at a maximum value. This lime dose varies from 0.3% CaO in some liquors to 1.2% CaO in others. With regard to the second question, the constant-condition method for laboratory carbonatation experiments has been described fully in the literature.[2]

D. E. TIPPENS (Amstar): I thought it very interesting that you compared the removal of impurities by phosphatation with that by membrane filtration and showed that, other than with color, these two processes are practically identical. Carbonatation and phosphatation are both crystallization processes, and they are both adsorption processes. You showed that carbonatation removes more sulfate than phosphatation does. How do you explain this difference? Would you elaborate, also, on the comparison of mem-

[1] Bennett, M. C. 1967. Liquor carbonatation. 1. Impurity effects on filterability. Int. Sugar J. 69: 101–104.

[2] Bennett, M. C., and Gardiner, S. D. 1968. Liquor carbonatation. 3. Laboratory procedures for comparing the quality of liquor or lime samples. Int. Sugar J. 70: 135–137, 173–176.

brane filtration with phosphatation as color or other impurity removal processes?

M. C. BENNETT: Our view is that phosphatation and membrane filtration are essentially removing the same impurities, defined by size. In membrane filtration you are straining them out according to the size of the holes in your Millipore membrane and in phosphatation you are grabbing them by flocculation. By comparing phosphatation with filtration through a series of Millipore membranes, you conclude that phosphatation is capable of grabbing particles down to about the same size that Millipore membranes of 0.1 μm will strain out.

D. E. TIPPENS: To return to Henry Gerstner's comments about the combining of the two processes: why should phosphatation and carbonatation not be synergistic?

M. C. BENNETT: Carbonatation alone does most of the job; the impurities do end up inside the chalk crystals. We saw in those early experiments that when we were just using chalk as a filter medium the impurities came pouring through, so in carbonatation we know very well that they must be held inside the chalk crystal. However, if the impurities have no chemical reason to go inside the chalk crystal, they won't go: that is why starch does not go inside. But, if there is any sort of acidic character in the impurity which is strong enough to form a weak calcium linkage, i.e., anything capable of forming a sparingly soluble calcium salt, it is likely to end up inside that chalk crystal. That's why, I believe, the strongly anionic color gets tucked away inside the chalk, because it is hooked to a calcium ion which is already part of the calcium carbonate lattice. Can you imagine what happens to that lattice when a color molecule gets in there? It is no wonder it is distorted out of all recognition. The color molecules are occupying the place that ought to be occupied by a carbonate ion.

M. K. FAVIELL (B. C. Sugar): If your raws came from widely varied points of origin, which of the two systems would have the advantage?

M. C. BENNETT: If you take Talofloc and Taloflote, the new chemicals, into consideration, there probably isn't very much to choose between the processes. In carbonatation you always have flexibility. You can turn up the lime dose, raise the temperature in the first tank and add some water to the melter to lower the Brix, not only for viscosity reasons, but also to improve the quality of chalk. You can use these changes to adjust the crystallization processes to suit an extra burden and thus adapt the process to many raw sugar types. You never had that flexibility in phosphatation until we found the chemicals that take over from the natural variation. But now in phosphatation, you can control the behavior of the system by adjusting the dose of cationic surfactant or polyacrylamide flotation agent. So I think the answer today is that there really isn't any difference between them. The answer 5 years ago would have been carbonatation, because of its greater flexibility of control.

CALCIUM ACTIVITY IN PHOSPHATE PRECIPITATION

By Margaret A. Clarke[1] and Frank G. Carpenter[2]
(Presented by Margaret A. Clarke)

ABSTRACT

The activity of calcium ion was measured, with the calcium-ion-selective electrode, in calcium phosphate precipitation processes in sugar solutions under varying conditions approximating those of refinery clarifiers. Critical ion activities were plotted by computer to determine the nature of the precipitate. Results do not indicate any of the precipitates usually ascribed to this process.

INTRODUCTION

The calcium phosphate clarification process for sugar liquors, the nature of the calcium phosphate precipitate, and the conditions, such as concentration and pH, which control that nature have been the subjects of much laboratory and refinery research (*2, 4, 6, 9–11, 14, 15*).[3] Every refinery that uses the process has its own particular optimal conditions and corrective procedures for faulty operation; however, these differ greatly in various refineries. The differences in methods of treatment of the same process emphasize the lack of basic information about the reactions taking place, most notably the lack of data at the molecular level.

All previous work on calcium phosphate precipitates has used some measure for total calcium concentration, e.g., EDTA titration or atomic absorption spectroscopy (*4, 9–11, 15*). In the present study, a new research tool for solution chemistry is used: the ion-selective electrode for calcium ion. This electrode, the first practical tool for activity measurement, measures the activity of calcium ion in solution. The results from measurement of activity or effective concentration should elucidate some of the chemical processes taking place during clarification.

[1] Administrator, Cane Sugar Refining Research Project, Inc., P. O. Box 19687, New Orleans, La. 70179.

[2] Research leader, Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, New Orleans, La. 70179.

[3] Italic numbers in parentheses refer to items under "References" at the end of this paper.

THEORY

It is well known that different calcium phosphates form under different conditions. Table 1 shows the different types of calcium phosphates and the Ca/P ratio for each.

The precipitate in refinery clarifiers has been variously identified as tricalcium phosphate (*12*), octacalcium phosphate (*3*), hydroxyapatite (*3*), and most frequently, as amorphous calcium phosphate (*1*). Basic information about which precipitate(s) forms under any particular set of refinery conditions will enable clarifiers to be operated at optimal efficiency, with selective removal of undesirable matter.

TABLE 1.—*Calcium phosphates*

Phosphate	Ca/P ratio
Hydroxyapatite, $Ca_{10}(OH)_2(PO_4)_6$	1.67
Tricalcium phosphate, $Ca_3(PO_4)_2$	1.5
Octacalcium phosphate, $Ca_8H_2(PO_4)_6$	1.33
Amorphous calcium phosphate	1.3–1.7
Dicalcium phosphate, $CaHPO_4$	1.0
Monocalcium phosphate, $Ca(H_2PO_4)_2$	.5

EXPERIMENTAL CONDITIONS

Many of the earlier workers focused on the type of precipitation at equilibrium and on the nature of the precipitate in solution after many hours, days, or even years. Most of this work used aqueous solutions with no sucrose present. In the current study, an attempt was made to approximate refinery clarifier conditions, using 60-Brix sucrose solution, rapid precipitation, and a 15- to 20-minute holding time for the precipi-

tate in solution. Only the temperature (room temperature) was not at refinery conditions, and it is recognized that this could be a critical difference.

Solutions of calcium nitrate (0.1 *M* $Ca(NO_3)_2$ in 60-Brix sucrose solution) and potassium dihydrogenphosphate (0.1 *M* KH_2PO_4 in 60-Brix sucrose solution), plus sufficient KOH pellets to give the desired final pH, were mixed rapidly, then stirred rapidly at room temperature for 20 minutes. The suspension was centrifuged for 20 minutes at 27,000 *g*. The filtrate was decanted and the precipitate was examined under a light microscope. The filtrate was analyzed for pH and pCa with a pH and a calcium-ion-selective electrode for the tests, respectively. It was also analyzed for total calcium concentration by an EDTA titration method (*13*) and for total phosphate concentration by the spectrophotometric molybdenum blue test (*8*). The procedure is outlined in figure 1.

pCa is here defined as the negative log of the activity of calcium ion, in moles per liter. The pH was varied from 5.45 to 10.05 to include the full range of formation of the various basic calcium phosphates. The order of mixing calcium and phosphate was varied. The speed and duration of stirring were kept constant, as were the centrifuging conditions.

Calcium-Ion-Selective Electrode

The calcium-ion-selective electrode is the differentiating element in this study. The Orion[4] calcium-ion-selective electrode, model 92-20, diagramed in figure 2, was used in this work for the pCa determination. The electrode (*7*) measures the activity of calcium ion in solution, i.e., the level of ionized, unbound calcium ion. The EDTA and other "total calcium" methods determine the total concentration of calcium in solution, whether the calcium ion is free or complexed. An earlier report (*5*) describes the behavior of this calcium electrode in sugar solutions. Many of the problems with its use can be solved by careful grounding and shielding. The electrode experimental setup is diagramed in figure 3. A saturated calomel fiber-junction reference electrode was used. The electrodes, along with a pH electrode, were connected through an electrode

[4] Orion Research Inc., 11 Blackstone St., Cambridge, Mass. $200.

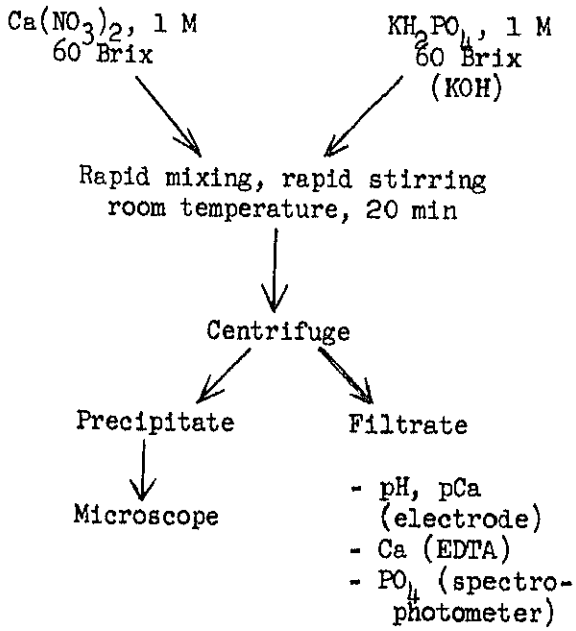


FIGURE 1.—Experimental procedure.

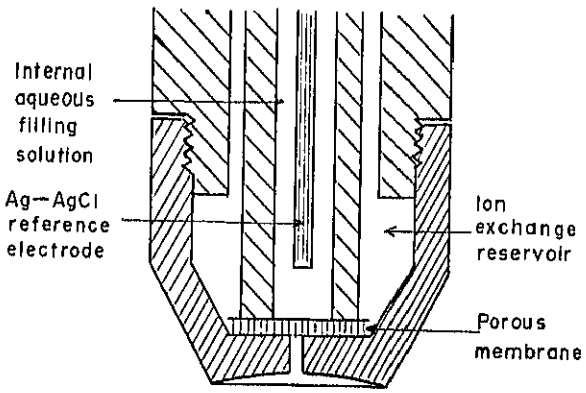


FIGURE 2.—Construction of liquid ion-exchange electrode.

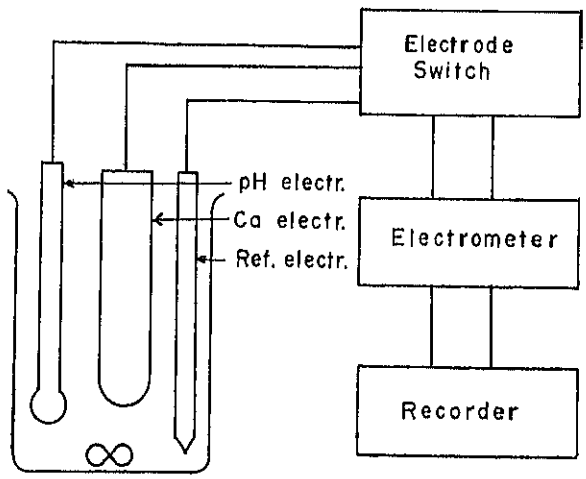


FIGURE 3.—Calcium electrode experimental setup.

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Monocalcium phosphate, $Ca(H_2PO_4)_2$	.5

EXPERIMENTAL CONDITIONS

Many of the earlier workers focused on the type of precipitation at equilibrium and on the nature of the precipitate in solution after many hours, days, or even years. Most of this work used aqueous solutions with no sucrose present. In the current study, an attempt was made to approximate refinery clarifier conditions, using 60-Brix sucrose solution, rapid precipitation, and a 15- to 20-minute holding time for the precipi-

tate in solution. Only the temperature (room temperature) was not at refinery conditions, and it is recognized that this could be a critical difference.

Solutions of calcium nitrate (0.1 *M* $Ca(NO_3)_2$ in 60-Brix sucrose solution) and potassium dihydrogenphosphate (0.1 *M* KH_2PO_4 in 60-Brix sucrose solution), plus sufficient KOH pellets to give the desired final pH, were mixed rapidly, then stirred rapidly at room temperature for 20 minutes. The suspension was centrifuged for 20 minutes at 27,000 *g*. The filtrate was decanted and the precipitate was examined under a light microscope. The filtrate was analyzed for pH and pCa with a pH and a calcium-ion-selective electrode for the tests, respectively. It was also analyzed for total calcium concentration by an EDTA titration method (*13*) and for total phosphate concentration by the spectrophotometric molybdenum blue test (*8*). The procedure is outlined in figure 1.

pCa is here defined as the negative log of the activity of calcium ion, in moles per liter. The pH was varied from 5.45 to 10.05 to include the full range of formation of the various basic calcium phosphates. The order of mixing calcium and phosphate was varied. The speed and duration of stirring were kept constant, as were the centrifuging conditions.

Calcium-Ion-Selective Electrode

The calcium-ion-selective electrode is the differentiating element in this study. The Orion[4] calcium-ion-selective electrode, model 92-20, diagramed in figure 2, was used in this work for the pCa determination. The electrode (*7*) measures the activity of calcium ion in solution, i.e., the level of ionized, unbound calcium ion. The EDTA and other "total calcium" methods determine the total concentration of calcium in solution, whether the calcium ion is free or complexed. An earlier report (*5*) describes the behavior of this calcium electrode in sugar solutions. Many of the problems with its use can be solved by careful grounding and shielding. The electrode experimental setup is diagramed in figure 3. A saturated calomel fiber-junction reference electrode was used. The electrodes, along with a pH electrode, were connected through an electrode

[4] Orion Research Inc., 11 Blackstone St., Cambridge, Mass. $200.

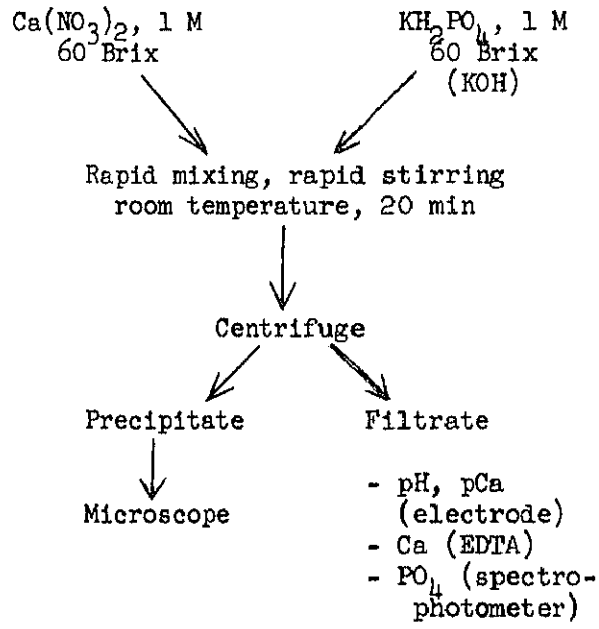

FIGURE 1.—Experimental procedure.

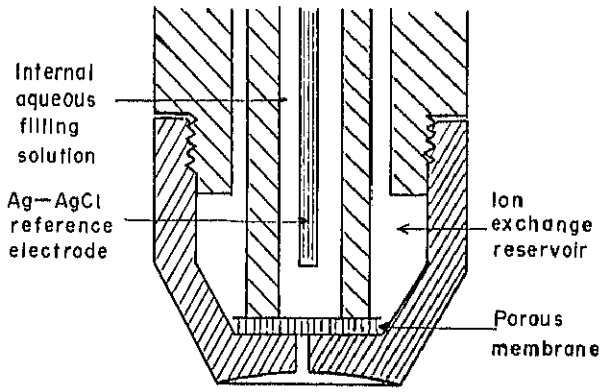

FIGURE 2.—Construction of liquid ion-exchange electrode.

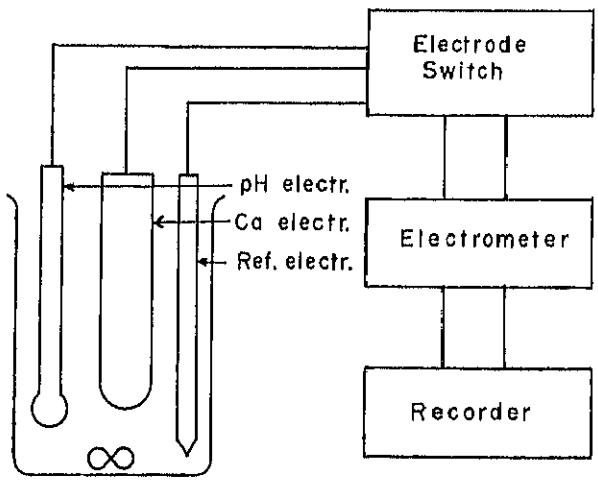

FIGURE 3.—Calcium electrode experimental setup.

switch[5] to an expanded-scale pH meter[6] which served as electrometer. The use of the electrode switch, which holds up to six pairs of electrodes, allows concurrent measurements of pH and pCa. The output from the electrometer was displayed on a strip chart recorder.[7]

Calibration of Electrodes

Calibration of the electrodes was done in 60-Brix sugar solutions, not in aqueous sugar-free solutions, since the calcium electrode gives quite a different slope in sugar solutions than in water alone (fig. 4). Calibration must be frequent: A one-point daily check is advisable, with a weekly six- or eight-point calibration, ranging from 10^{-1} to 10^{-6} M calcium ion. Calibration is at room temperature, with reference electrode, stirring speed, and solution depth all held constant. Age changes the slope of the calibration plot slowly, but the intercept rapidly.

Figure 5 shows a series of typical calibration plots. Computer programs were developd to calculate the calcium activity from the amount of reagents used in making the standard calcium solutions. The dielectric constant and ionic strength of the solution were first calculated from the amounts of reagents and sugar used. These were then used in the Debye-Huckel approximation to calculate activity coefficients,

[5] Orion model 605.
[6] Corning model 12.
[7] Honeywell model Electronik 194.

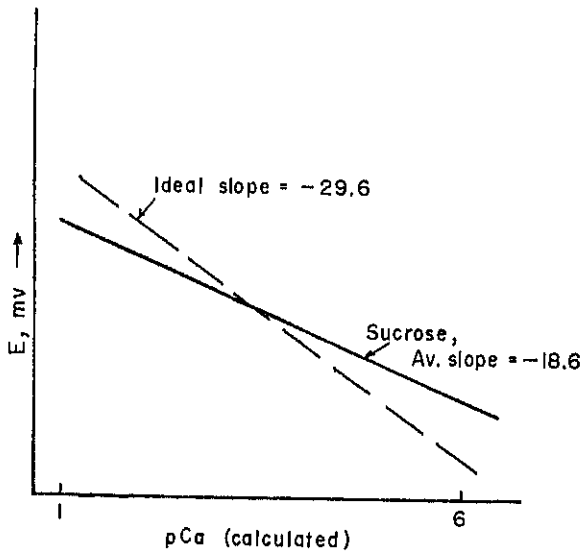

FIGURE 4.—Calibration of calcium electrode in water and in 60-Brix sucrose.

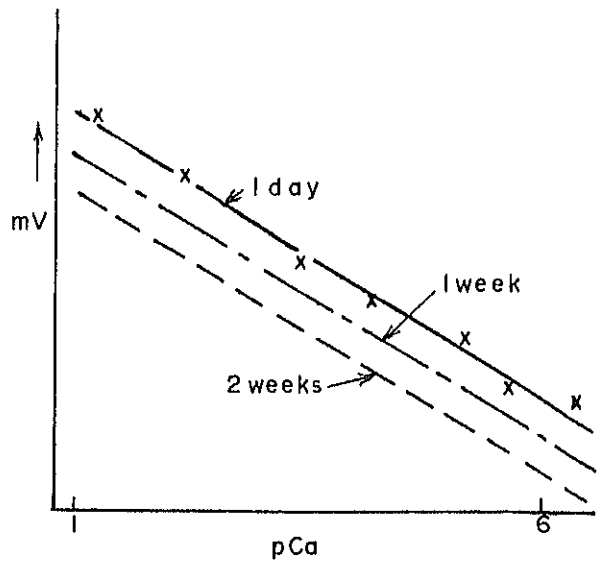

FIGURE 5.—Calcium electrode calibration plots, 60-Brix sucrose.

and from these, calcium activity. The output of the computer was a plot of millivolts vs. theoretical pCa with, theoretically, a Nernstian slope. A millivolt reading from electrodes in a mother liquor could be directly translated into a pCa reading by use of this calibration plot.

Calculation of Data

A plot of critical ion activities has been developed by MacGregor and Brown (*10*) and others, which plots the activity of calcium in mother liquor against phosphate concentration, such that the slope of the plot gives the Ca/P ratio at precipitation. In all previous work (*10, 11, 15*) activity coefficients have been calculated by Debye-Huckel and modified Debye-Huckel methods. In this work a direct measure of calcium ion activity was used instead.

The precipitation of calcium phosphates can be written in the following general form:

$$x\mathrm{Ca(OH)_2}+y\mathrm{H_3PO_4}\rightarrow \mathrm{Ca_xH(}y-2x\mathrm{)(PO_4)}y +2x\mathrm{H_2O}.$$

The solubility product constant for this relationship is

$$K_{sp}=[\mathrm{Ca(OH)_2}]^x\cdot[\mathrm{H_3PO_4}]^y$$

and $\log K_{sp}=x\log[\mathrm{Ca(OH)_2}]+y\log[\mathrm{H_3PO_4}]$.

Rearranging the equation gives

$$-\log[Ca(OH)_2] = \frac{-\log K_{sp}}{x} - \frac{y}{x}[-\log (H_3PO_4)]$$

Writing the negative logs in the p format gives

$$p[Ca(OH)_2] = pK_{sp}/x - (y/x)\,p(H_3PO_4)$$

or $$p\text{Ca} + 2p\text{OH} = k - y/x\,(p\text{H} + p\text{H}_2\text{PO}_4).$$

Thus, a plot of (pCa+2pOH) versus (pH +pH$_2$PO$_4$) for any set of conditions will give a graph with slope y/x, the inverse Ca/P ratio of the precipitate being formed. This ratio is indicative of the nature of the precipitate formed. Computer programs have been devised in this study to calculate fractions of all species present and plot the critical ion activities. An outline of the program follows:

Input:—pH, pCa (measured or practical, activity of calcium ion), total calcium, total phosphate, temperature, Brix.

Calculation: (1) Dielectric constant is calculated from sugar concentration. (2) Ionic strength is assumed. (3) Debye-Huckel activity coefficients for all species except Ca^{++} and H^+ are calculated; for these the measured activities were used. (4) Fractions of all phosphate species present, including ion pairs, are calculated with the usual equations for polybasic acids and the published ionization constants. (5) Ionic strength is calculated from quantities of the species present. (6) The program is iterated around ionic strength until sufficient precision is attained.

Output: Plot of critical ion activities.

RESULTS

The plot of critical ion activities for the data from this study is shown in figure 6. The inverse slope of the line—the Ca/P ratio—is 0.55. This ratio is below that for hydroxyapatite (1.67), tricalcium phosphate (1.5), and octacalcium phosphate (1.33), and is out of the amorphous calcium phosphate range (1.3–1.7) (table 1); it is in the area of the monocalcium phosphate ratio (0.5), but this precipitate has not previously been observed in this pH range. At this point it must be emphasized that these are the first such results calculated with an experimental activity measurement.

Table 2 shows the data used in these calculations. These data were examined for trends in

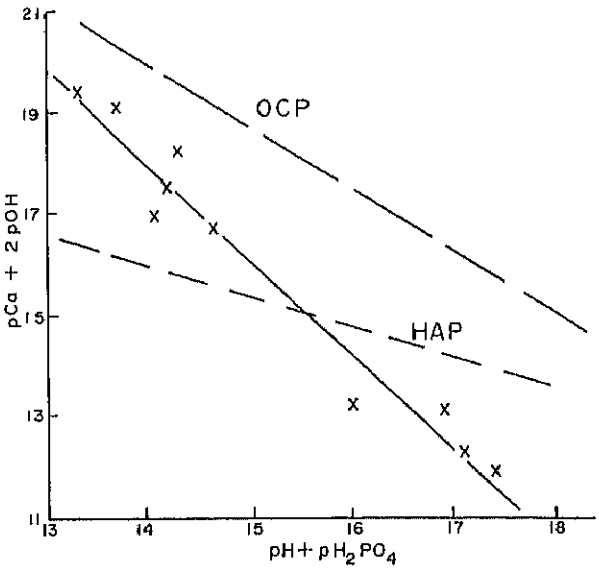

FIGURE 6.—Plot of critical ion activities.

TABLE 2.—*Experimental data*

pH	pCa	pTCa	pPO$_4$
5.45	2.30	2.45	2.86
6.40	3.92	3.24	2.34
6.70	2.93	3.13	2.58
6.87	3.92	3.26	2.52
6.98	2.94	2.97	2.00
7.43	3.57	3.28	2.17
8.94	2.98	3.70	2.05
9.55	4.15	3.51	2.44
9.90	3.98	3.29	2.21
10.05	3.86	3.70	2.48

the relationship of the calcium ion activity to other factors. The solution pH becomes more basic with decreasing total phosphate content of the solution, as would be expected. There is no discernible trend in the relationship between calcium ion activity and total calcium other than a correlation with pH (and there are exceptions to that correlation). At low pH, there is a tendency for the activity of calcium to be lower than the total calcium concentration, whereas at high pH the tendency is the reverse. Surprisingly, there was no apparent dependence on the order of mixing solutions. Carpenter and coworkers (*1*) reported that the nature of the precipitate initially formed was strongly dependent on the concentration of reagents. This observation indicates an initial kinetically controlled reaction, rather than the thermodynamically controlled reaction, in operation at equilibrium. A dependence on order of mixing would, therefore, have been expected.

In the high pH range, an amorphous gellike precipitate formed; it showed no crystalline nature under the light microscope up to 50 × magnification. At low pH's, a fine needlelike precipitate appeared. The pH in this case was 5.45. These crystals could not be hydroxyapatite or octacalcium phosphate; they do resemble anhydrous dicalcium phosphate (DCPA). Over a period of 18 hours, after decantation of the mother liquor, this precipitate lost its crystalline nature and became an amorphous gel; this is the reverse of the usual transformation from amorphous calcium phosphate into hydroxyapatite. The precipitate might be DCPA, or possibly a coprecipitated mixture of some calcium phosphate and sucrose.

Future plans for this study include analyses of the various precipitates, by chemical and physical methods, and comparison of the critical activities plot with that using calculated activity coefficients for the same data.

REFERENCES

(1) Bhangoo, M. S., and Carpenter, F. G. 1966. Precipitation of calcium phosphate. Proc. 1966 Tech. Sess. Cane Sugar Refining Res., pp. 125–136.

(2) Brecevic, L., and Furedi-Milhofer, H. 1972. Precipitation of calcium phosphates from electrolyte solutions. II. The formation and transformation of the precipitates. Calcif. Tissue. Res. 10: 82–90.

(3) Brown, W. E. 1964. Crystal chemistry of calcium phosphates. Proc. 1964 Tech. Sess. Cane Sugar Refining Res., pp. 26–33.

(4) Childs, C. W. 1969. Equilibria in dilute aqueous solutions of orthophosphates. J. Phys. Chem. 73: 2956–2960.

(5) Clarke, M. A. 1970. The effect of solution structure on electrode processes in sugar solutions. Proc. 1970 Tech. Sess. Cane Sugar Refining Res., pp. 179–188.

(6) Dickens, B., Bowen, J. S., and Brown, W. E. 1971. A refinement of the crystal structure of $CaHPO_4$ (Snythetic monetite). Acta Cryst. B28: 797–806.

(7) Durst, R. A. (ed.). 1969. Ion-selective electrodes. Nat. Bur. Stand. (U.S.) Spec. Publ. 314.

(8) Gee, A., Domingues, L. P., and Deitz, V. R. 1954. Determination of inorganic constituents in sucrose solutions. Anal. Chem. 26: 1487–1491.

(9) Kukura, M., Bell, L. C., Posner, A. M., and Quirk, J. P. 1972. Radioisotope determination of the surface concentrations of calcium and phosphorus on hydroxyapatite in aqueous solution. J. Phys. Chem. 76: 900–904.

(10) MacGregor, J., and Brown, W. E. 1965. Blood: bone equilibrium in calcium homeostasis. Nature (Lond.) 205: 359–361.

(11) McDowell, H., Brown, W. E., and Sutter, J. R. 1971. Solubility study of calcium hydrogen phosphate ion-pair formation. Inorg. Chem. 10: 1638–1643.

(12) Meade, G. P. 1963. Cane sugar handbook. 9th ed. p. 328. John Wiley & Sons, New York.

(13) ———. 1963. Cane sugar handbook. 9th ed. p. 606. John Wiley & Sons, New York.

(14) Termine, J. D., and Eaves, E. D. 1972. Comparative chemistry of amorphous and apatitic calcium phosphate preparations. Calcif. Tissue Res. 10: 171–197.

(15) Walton, A. G., Bodin, W. J., Furedi, H., and Schwartz, A. 1967. Nucleation of calcium phosphate from solution. Can. J. Chem. 45: 2695–2701.

DISCUSSION

S. STACHENKO (Redpath): What would the critical activities plots look like if made with the Debye-Huckel activity coefficients?

M. A. CLARKE: We have not yet made the plot using Debye-Huckel coefficients, but plan to do so shortly, since Debye-Huckel coefficients can be calculated readily from our data. I think that plot would probably give a slope similar to literature values because literature work is based on this type of calculation.

S. STACHENKO: I would like also to comment on the fact that we tried, about 4 or 5 years ago, to use the calcium electrode and had many of the problems you mention. I noticed how much care you took in the grounding and shielding—this seems to help solve some of the problems. Do you feel that the electrodes that are being produced now are of better quality than the ones made 4 or 5 years ago?

M. A. CLARKE: I have been working with these electrodes for about 4 years, and I have not found much change in them during that time. We use only the Orion liquid electrode; we tried solid-type electrodes put out by other manufacturers and we found them to be much poorer.

S. STACHENKO: We also tried the solid forms first, and we found them very unsatisfactory.

G. W. MULLER, JR. (Kerr-McGee): We are quite interested in the use of the calcium electrodes but at a much higher temperature—up in the 80° C range. What difficulties would we have at these temperatures?

M. A. CLARKE: You would have a terminal difficulty in that case, because over about 60° C the ion-exchange liquid runs through the membrane and out of the electrode. You cannot use it over 60° C.

R. S. PATTERSON (California and Hawaiian): You have indicated error in the electrode readings; do you think that affects the data significantly?

M. A. CLARKE: I had expected that it would affect the graph that we got, so we assumed the maximum error that could come from the electrode and made a computer plot using this error. It made very little difference. The only thing that seems to affect that plot to any significant degree is the change in pH. A change in the electrode reading of 0.1 pCa made a difference in the slope of only about one-tenth unit.

R. S. PATTERSON: I suppose frequent calibration of the electrodes helps to minimize their error.

M. A. CLARKE: Yes, it is very necessary, especially for use in sugar solutions.

M. C. BENNETT (Tate & Lyle): Would you tell us a little more about those activity measurements. For instance, have you in the course of this work studied the effects of sucrose concentration on calcium activity, in calcium salt solutions? These data are sadly missing from the literature. I would be very glad to know what happens in the Brix ranges which we use in process liquors. There isn't enough water for the sucrose and when you put calcium ions into solution, there must be even more competition for the limited amount of water available. What effect does that have on pCa?

M. A. CLARKE: The addition of sucrose has a considerable effect on pCa. The electrode will give a fairly Nernstian slope—around 29 mV/pCa in calcium ion solutions containing no sucrose. But, in the 60-Brix range the slope goes down to 18 or 19 mV/pCa. That was taken into account on the calibration procedure. I agree with you about the importance of the storage of water. The activity of water in the sucrose-water mixture which is the solvent for the calcium phosphate is a controlling factor in the precipitation.

G. P. MEADE (retired): Was all this work done at room temperature?

M. A. CLARKE: Yes.

G. P. MEADE: You suggest that you will do work at higher temperatures but there is a good deal of disagreement as to what temperature should be used for practical work. Also, in line with Dr. Bennett's remark, what should the Brix of the solution be? It can sometimes go as high as 65 to 67. Did you work at these Brix? Would you be able to use these conditions in your methods?

M. A. CLARKE: We could work at higher Brix, but the temperature will be limited by the calcium electrode. It cannot be used over 50° C satisfactorily.

G. P. MEADE: There is a process by Saranin[1] which uses 80° C in the main operation, with a secondary heating at 95° which is said to be extremely practical.

M. A. CLARKE: We can't work at these temperatures with the electrodes as they are now.

[1] Saranin, A. P. 1972. Technology of phosflotation of sugar melt. Sugar Technol. Rev. 2: 1–72.

EVALUATION OF FLOCCULANTS IN REFINERY MELT LIQUOR AND SCUM

By James C. P. Chen and Robert W. Picou[1]
(Presented by James C. P. Chen)

ABSTRACT

Synthetic polymers have replaced the natural flocculants (algin, soybean extract, gum arabic, etc.) used in the sugar industry for clarification purposes. Results on the performance of different polymer flocculants in raw melt liquor clarification, scum conditioning, muds conditioning, and sediment and scum filtration showed that the same flocculants were best for all the different applications. The leading flocculants were all anionic. The optimum dosage varied widely.

INTRODUCTION

As in the application of polymer flocculants to raw juice clarification, good results have been achieved with their application to cane mud filtration.[2] Several types of polymers have been tried in refinery raw melt phosphofloatation processes, and some refineries have already initiated flocculants into their regular operation,[3] though the practice is still not as common as in raw juice clarification. Because so many different types of polymers are recently available, we were interested in looking at them more closely to find out their characteristics and comparative effectiveness.

It is understood that charge neutralization is not the whole mechanism of flocculation, but functions rather as an aid to other mechanisms such as "bridging." Although most of the commercialized polymers for sugar solutions are anionics, bridging can also take place with cationics.

MATERIALS AND METHODS

For both raw melt liquor and scum, we have tried some anionics, some cationics, and some nonionics.

Raw Melt Liquor

Samples were collected from the blowup tank where liquor at 150° to 155° F, 58 to 60 Brix, is treated with phosphoric acid and lime.

A row of graduated measuring cylinders was used for each run. All polymers were prepared in 0.1% solution. The starting dosage was 8 p/m on solids, which is generally recommended by polymer producers and has been applied in refinery operation.[4] The dosage was then increased to as much as 80 p/m, to observe the characteristics of different polymers.

The first batch of polymers contained only anionics; some cationics and nonionics were added to the second batch of tests. Separan AP-30 is used as the reference flocculant, denoted as No. 1 (No. 0 is blank). The other flocculants are identified by number only.

The effect of each polymer is shown in figures 1–12. Each shows the residence time of the polymer and the dosage in parts per million. The order of the polymers in the cylinders is marked individually in each figure.

From the first batch of polymers, grouping by similar degree of effectiveness shows the following relative dosages:

Polymer	*Dosage*
No. 1	40 p/m
No. 2	80 p/m
No. 3	8 p/m

[1] Technical director and chief chemist, Southdown Sugars, Inc., Houma, La. 70360.

[2] Chen, J. C. P., and Picou, R. W. 1972. Polymer flocculants in mud filtration. Proc. Am. Soc. Sugar Cane Technol. (In press.)

[3] Wallenstein, H. M. 1970. Operating control of a phosphoric acid floatation clarifier. Sugar Ind. Technol. 23: 32–39.

[4] Chen and Picou, cited in footnote 2.

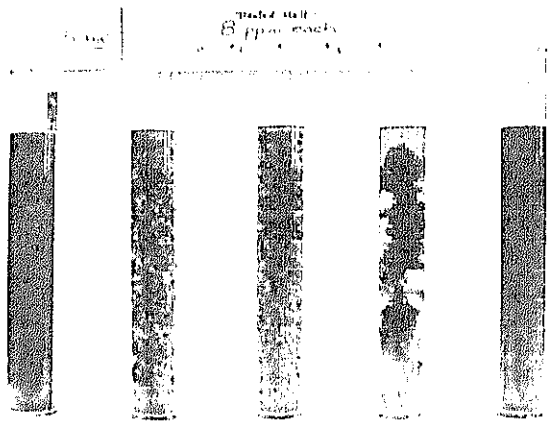

FIGURE 1.—Four polymers compared with blank (No. 0) at 8 p/m (raw melt liquor).

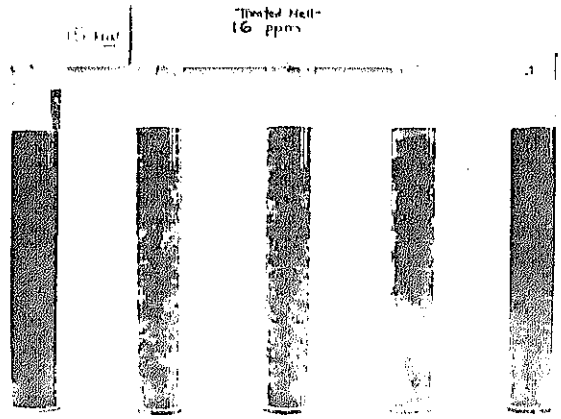

FIGURE 2.—Four polymers compared with blank (No. 0) at 16 p/m (raw melt liquor).

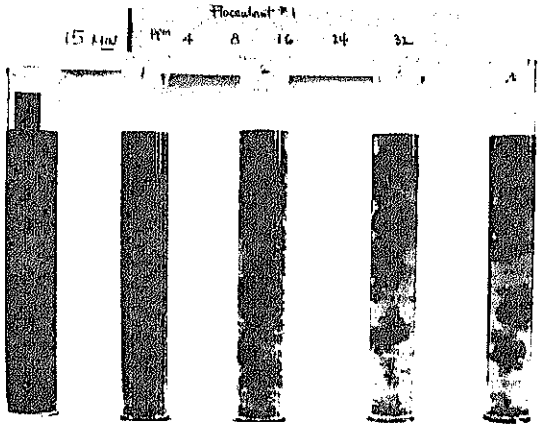

FIGURE 3.—Polymer No. 1 at different dosages (raw melt liquor).

From the second batch, the grouping shows:

$$\frac{\text{Z-6}}{\text{R}} > \text{No. 1} \ldots\ldots 8 \text{ p/m}$$

$$\frac{\text{Z-6}}{\text{R}} < \text{No. 1} \ldots\ldots 40 \text{ p/m}$$

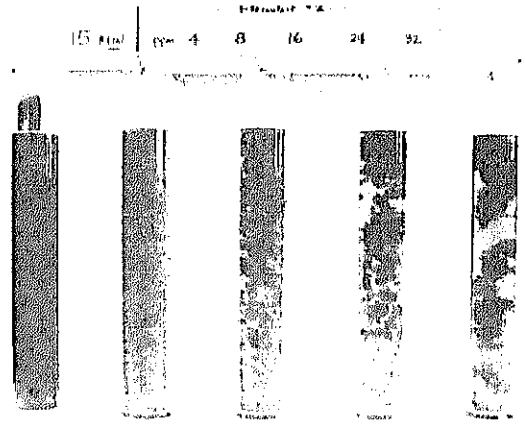

FIGURE 4.—Polymer No. 2 at different dosages (raw melt liquor).

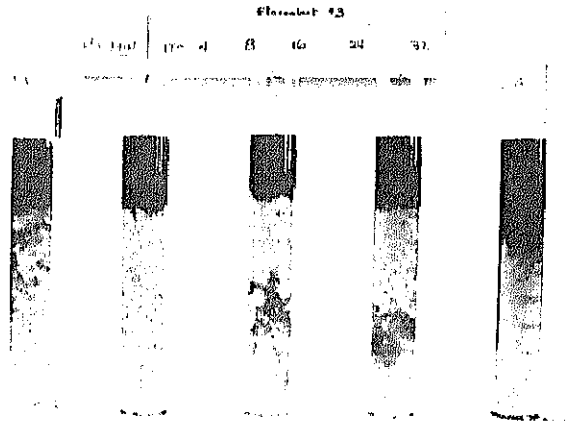

FIGURE 5.—Polymer No. 3 at different dosages (raw melt liquor).

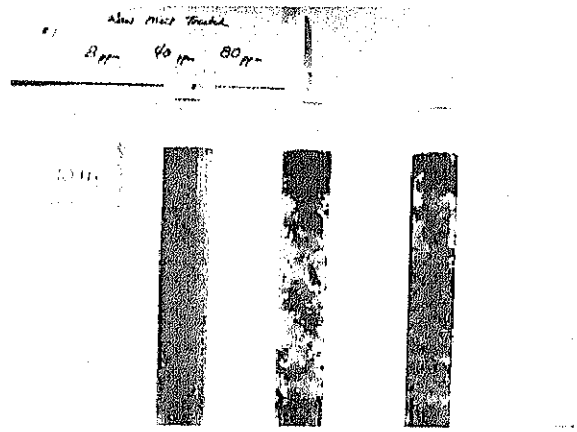

FIGURE 6.—Polymer No. 1 at high dosages (raw melt liquor).

The third batch of polymers consisted of No. 1, No. 3, Z–6, and R and was run with 8 p/m and 40 p/m dosages (not shown in figures).

The final grouping of comparable polymers, with good flocculation and clarity of liquor, is—

Polymer	*Dosage*
No. 3 (anionic)	8 p/m
Z-6 (anionic)	8 p/m
R (anionic)	8 p/m

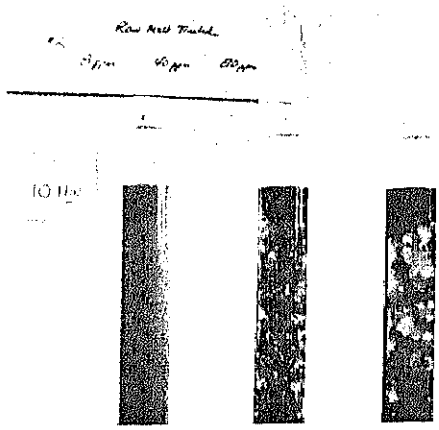

FIGURE 7.—Polymer No. 2 at high dosages (raw melt liquor).

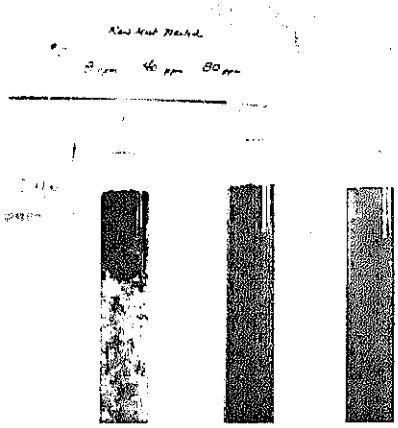

FIGURE 8.—Polymer No. 3 at high dosages (raw melt liquor).

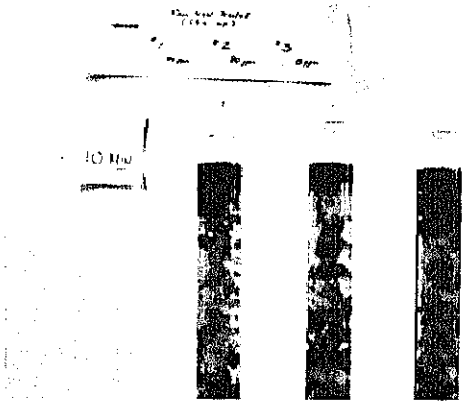

FIGURE 9.—Closeup of three polymers at different dosages but similar flocculation (raw melt liquor). No. 1, 40 p/m; No. 2, 80 p/m; No. 3, 8 p/m.

None of the cationics, nor the nonionics, is comparable with the three anionics shown above.

In order to see how these polymers behave in filtration, a series of filtration tests was carried

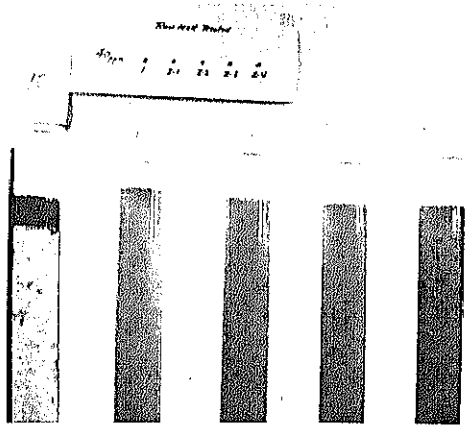

FIGURE 10.Polymers Z-series compared with No. 1 at 40 p/m (raw melt liquor).

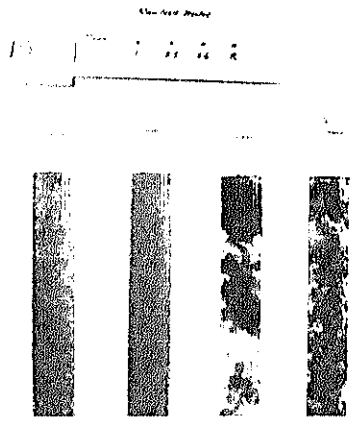

FIGURE 11.—Polymers Z-series and R compared with No. 1 at 8 p/m (raw melt liquor).

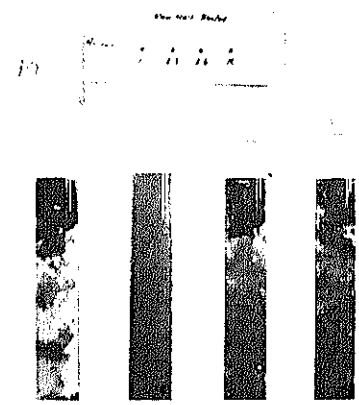

FIGURE 12.—Polymer Z-series and R compared with No. 1 at 40 p/m (raw melt liquor).

out with the same liquor for each series of different polymers. Results are shown in figure 13 for three different melt liquors. The conditions were as follows:

Polymer solution 0.1%
Raw melt liquor ..... 150–155° F
(treated from process)
Dosage of polymer . . 8 p/m on solid

The mixture was stirred for 30 seconds and allowed to stand for 60 seconds before filtration. Then, with 1 g filter aid, the mixture was filtered at a vacuum of 20 inches of mercury through a backing disk of Milk-Test.

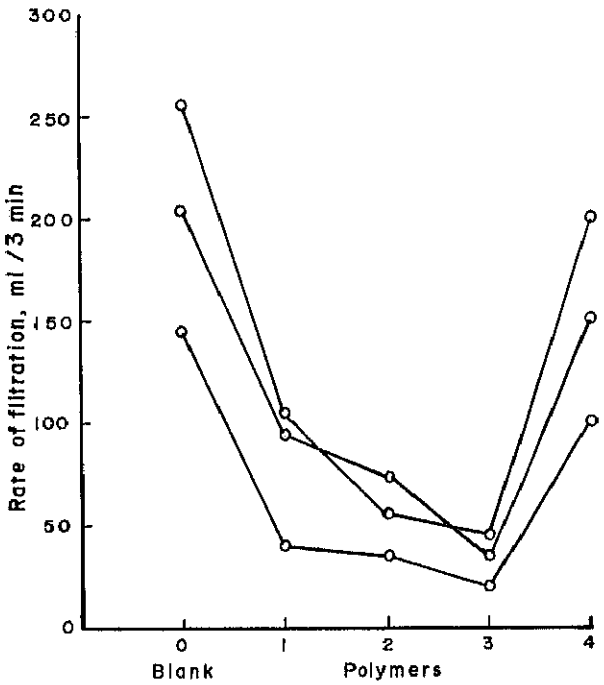

FIGURE 13.—Filtration of raw melt liquors with polymers.

Scum Conditioning

There are several ways to handle the clarifier scum.

1. *Secondary floatation.*—This was once tried by the authors at the clarifier station, by dosing a polymer solution into one of the scum troughs of a clarifier. The polymer solution was thereby brought into a receiving tank where scum was being diluted, and so mixed with the scum. The diluted scum containing the polymer was sent back to an assigned clarifier for secondary floation.

The flocculant did produce results superior to those without it. The process was discontinued because some sediment accumulated at the bottom of the clarifier, and it had to be liquidated frequently.

2. *Subsiding of scum and filtration of sediment.*—This was formerly practiced at Southdown Refinery. The scum was diluted and left for sedimentation. The supernatant liquid was separated out as sweetwater, and the sediment was filtered by a pressure filter.

The application of polymer to this type of treatment is similar to the application of polymer to raw cane juice clarification and mud conditioning. Tests on scum sedimentation were carried out by diluting the scum to 16–18 Brix at 135° F, and dosing with different polymers at different dosages to compare with Separan AP–30, denoted as polymer No. 1. The effectiveness of each polymer is shown in figures 14 to 21. Time, dosage, and polymer numbers are marked on the individual figures.

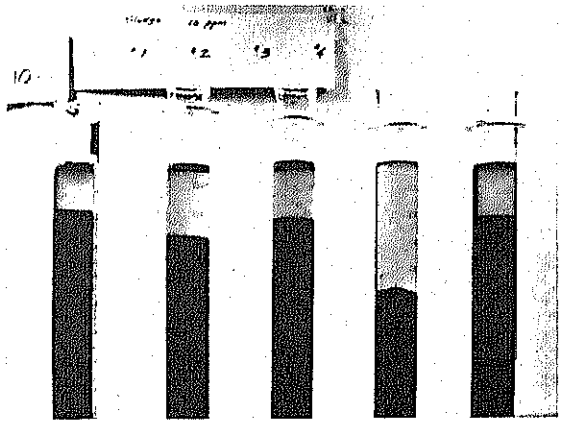

FIGURE 14.—Polymers No. 1 to No. 4 compared with blank (No. 0) at 10 p/m on solid (scum).

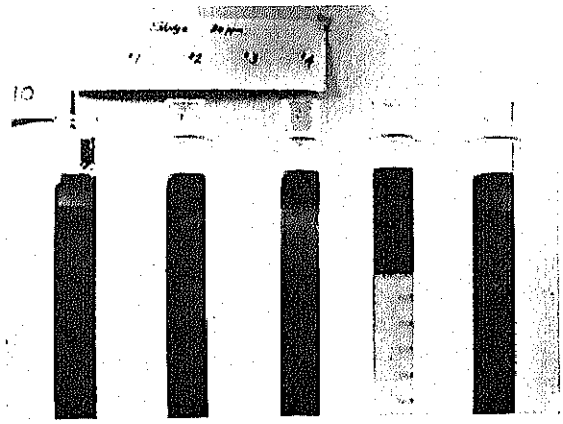

FIGURE 15.—Polymers No. 1 to No. 4 compared with blank (No. 0) at 30 p/m on solid (scum).

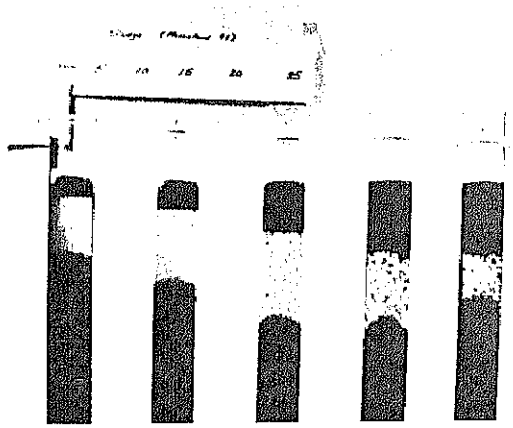

FIGURE 16.—Polymer No. 1 at different dosages (scum).

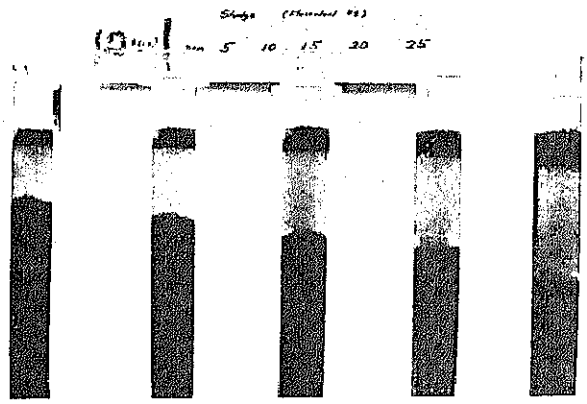

FIGURE 17.—Polymer No. 2 at different dosages (scum).

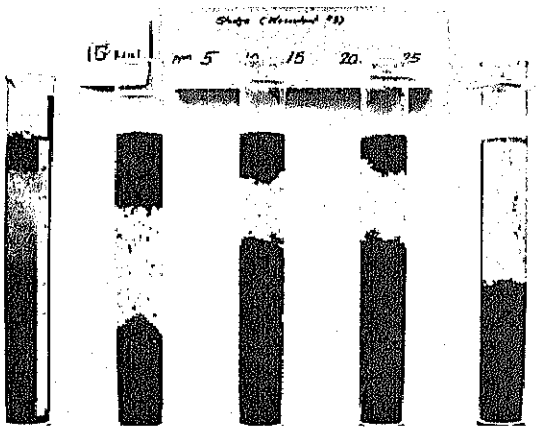

FIGURE 18.—Polymer No. 3 at different dosages (scum).

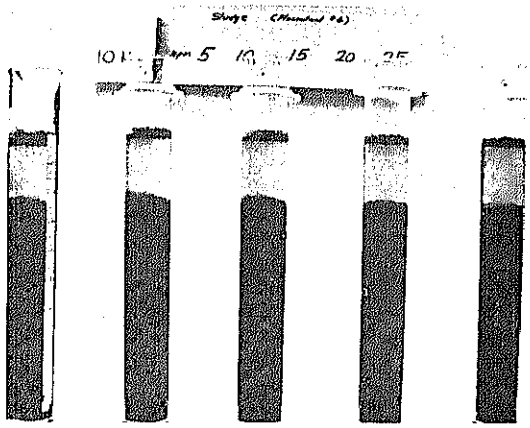

FIGURE 19.—Polymer No. 4 at different dosages (scum).

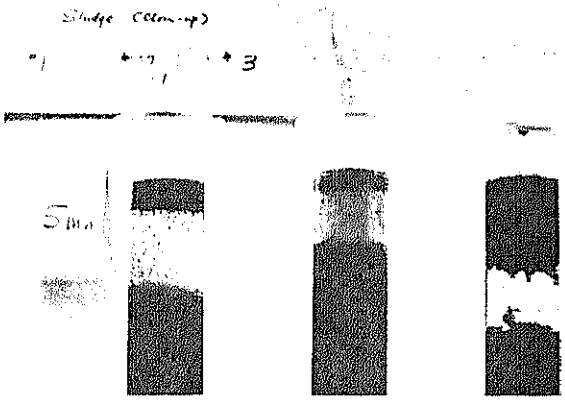

FIGURE 20.—Closeup of polymers No. 1, No. 2, and No. 3 at 20 p/m (scum).

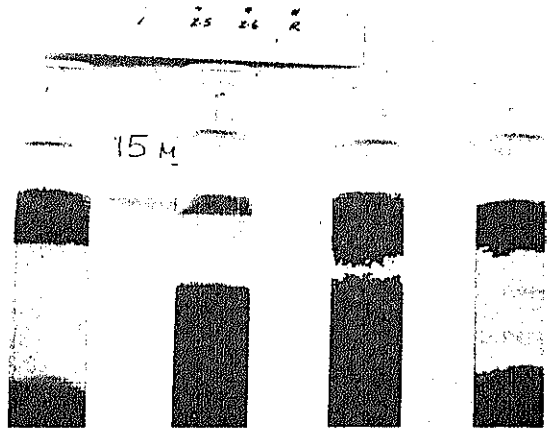

FIGURE 21.—Closeup of polymers No. 1, Z–5, Z–6, and R at 20 p/m (scum).

The first mixed batch of polymers leaves No. 4 out, and No. 3 gives the best performance (fig. 19). The second mixed batch of polymers leaves out Z–1, Z–2, Z–3, Z–4, and Z–5, with Z–6 and R performing best (fig. 20). The third mixed batch (not shown in figures) groups the leading performers from the two former batches together for a closeup, showing that No. 3 >Z–6 >R. The flocculation of these polymers is shown in figure 22, and the difference in clarity is evident.

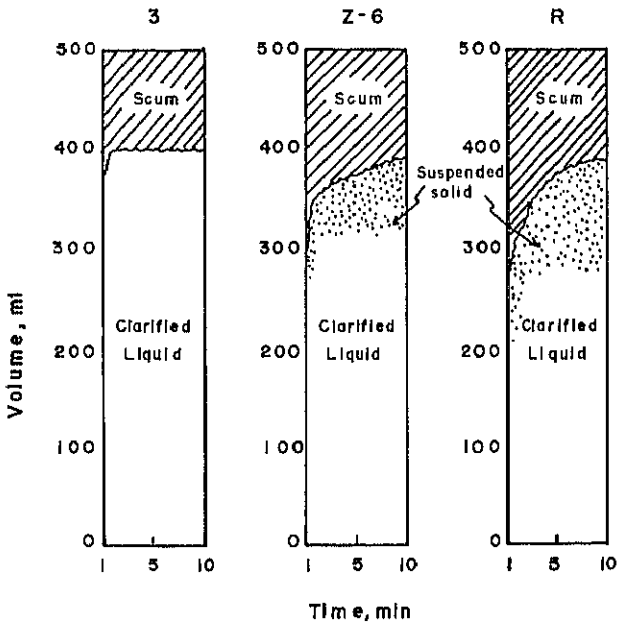

FIGURE 22.—Flocculation of scum. Polymers 20 p/m on solids.

This final comparison shows that those polymers leading in performance for scum are the same as those which work best in the raw melt liquor. All three are anionics. Among all that have been evaluated, none of the cationics nor any of the nonionics is comparable to any of these three anionics.

Muds Conditioning

Regarding the conditioning of sediment for filtration, our previous studies on mud conditioning in the raw sugar house are of interest. Figures 23 to 26 are micrographs taken with raw sugar house mud, with and without polymer treatments. The bridging effect of the polymer is shown in the micrographs, which were taken by a scanning electron microscope. Although the polymer could not be seen per se, the change in the surface appearance of the mud is clearly visible.

Sediment Filtration

Regarding the filtration of sediment,[5] our experience in mud filtration with vacuum drum filters is of interest. Figure 27 shows the design of the mud conditioning equipment. Tables 1 and 2 compare results from two new polymer flocculants with those from the reference standard Separan AP–30.

[5] Chen and Picou, cited in footnote 2.

FIGURE 23.—Sugar mud (5000 ×).

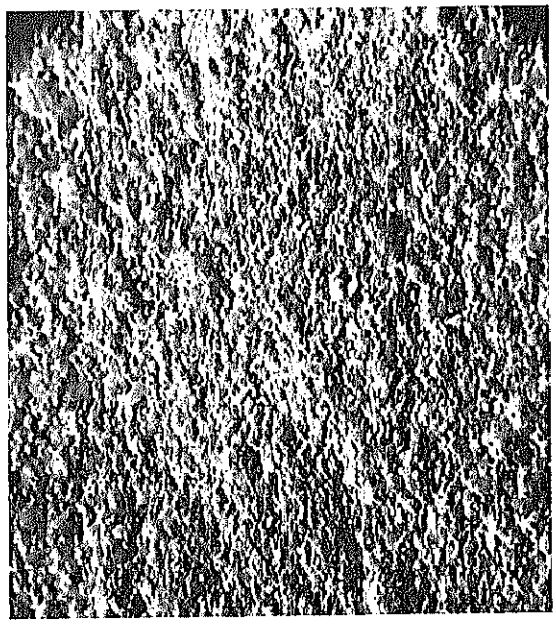

FIGURE 24.—Sugar mud (1,000 ×).

The bridging effect of flocculants on refinery scum can be seen from the micrographs in figures 28 to 31, taken by a scanning electron microscope.[6] These sediments appear quite different from those of cane juice mud, shown in the previous four micrographs, figures 24 to 27.

[6] Courtesy of Nalco Research Centre.

FIGURE 25.—Sugar mud + 6 p/m Nalco 41AO6 (1,000 ×).

FIGURE 26.—Sugar mud + 10 p/m Nalco 41AO6 (1,000 ×).

It may be noted here that polymers No. 1 and No. 2 are similar in appearance. Polymer No. 4 is different from the other three. Polymer No. 3 has a very attractive and impressive appearance, which could be related to its superior flocculation performance.

TABLE 1.—*Raw house mud filtration, Separan AP–30 vs. Percol LT–26 (average of several parallel duplications)*

1971 grinding season	Separan AP–30	Percol LT–26
Cake thickness .....cm....	1.09	0.94
Cake moisture .....%....	56.26	55.80
Cake pol	5.78	5.55
Cake weight (4"×5") .....g....	160.8	146.6
Dosage, 0.5% solution ....l/h....	204.3	147.3
Calculation:		
Wt. cake .....g/ft²....	1158	1056
Wt. solids .....g/ft²....	507	467
Wt. pol .....g/ft²....	67	59
Cake pol/100 solids	13.21	12.56
Cake specific wt. g/ft² cm....	1062	1123
Comparison:		
Capacity .....wt. solids....	100	92
Pol loss .....on solids....	100	95
Dosage	100	72
Specific wt.	100	106

TABLE 2.—*Raw house mud filtration, Separan AP–30 vs. Nalco 41AO6 (average of several parallel duplications)*

1971 grinding season	Separan AP–30	Nalco 41AO6
Cake thickness .....cm....	0.98	0.92
Cake moisture .....%....	53.68	52.30
Cake pol	6.21	5.59
Cake wt (4"×5") .....g....	165.4	153.2
Dosage, 0.5% solution ....l/h....	126.95	189.30
Calculation:		
Wt. cake .....g/ft²....	1191	1103
Wt. solids .....g/ft²....	552	526
Wt. pol .....g/ft²....	74	62
Cake pol/100 solids	13.41	11.72
Cake specific wt. g/ft² cm....	1215	1199
Comparison:		
Capacity .....wt. solids....	100	95
Pol loss .....on solids....	100	87
Dosage	100	[1]70–75
Specific wt.	100	99

[1] Converting into same cost basis.

Filtration of Scum

The scum is diluted to 15–20 Brix (refractometer) at 155° F, and dosed with different polymers at 30 p/m (on solids). It is then stirred 30 seconds, and allowed to stand for 60 seconds. Then 1 g of filter aid is added and mixed well, and the mixture is filtered through a backing disk of Milk-Test, under 20 inches of mercury vacuum for 3 minutes.

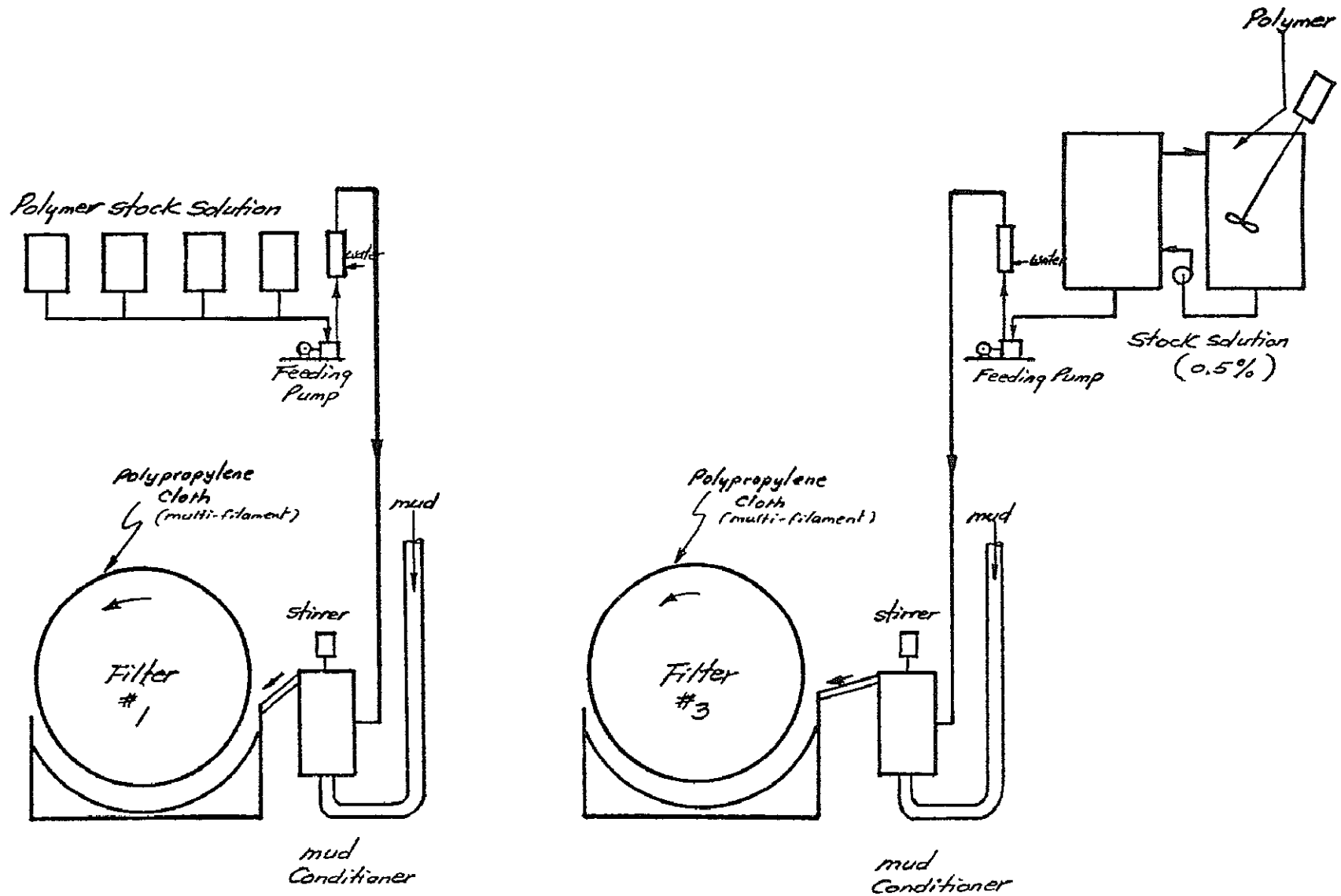

FIGURE 27.—Polymer dosing device. Raw house mud conditioning and filtration.

FIGURE 28.—Refinery scum with polymer No. 1 (900 ×).

FIGURE 30.—Refinery scum with polymer No. 3 (900 ×).

FIGURE 29.—Refinery scum with polymer No. 2 (900 ×).

FIGURE 31.—Refinery scum with polymer No. 4 (900 ×).

The average results of several series of tests are shown in figure 32. Setting the blank at 100 gives the following relative filtration rates:

Blank	100
Polymer No. 1	140
Polymer No. 2	180
Polymer No. 3	200
Polymer No. 4	100

It is interesting to note the reverse effect in this series of filtrations compared with the rates in the series of filtrations of raw melt liquor (fig. 13), where lower polymer dosages are applied to more highly concentrated liquors.

Molecular Weight of Polymers

The most effective flocculants are those of high molecular weight. The high-molecular-weight polymers produce solutions of high viscosity; therefore more time for preparing the stock solution is needed. Also, the particles of high-moleoular-weight polymers may form agglomerates of gel which are very difficult to dissolve.

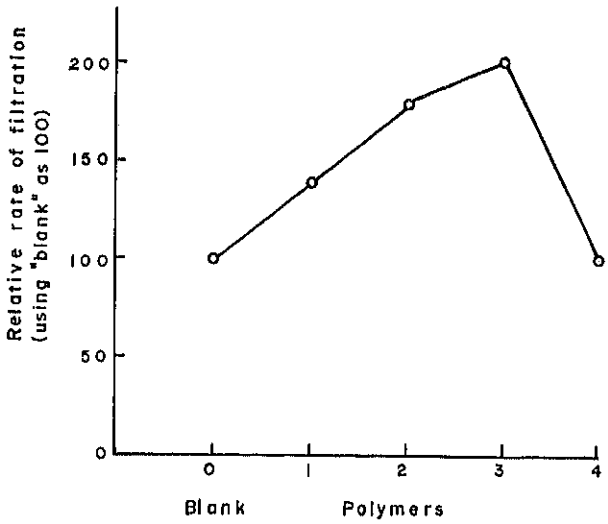

FIGURE 32.—Filtration of scum. 15–20 Brix. Polymer 30 p/m.

Of the polymers evaluated, those that are in liquid form gave no difficulty in preparing solutions. There are others in flake, granule, or dust forms. Some of them are very difficult to dissolve uniformly because of agglomerate formation; however, one of the leading polymers shows excellent solubility. It is understood that different techniques in polymerization may produce different configurations of molecules, and, thus, differences in solubility. Also, it has been learned that by formulating the polymers with some inorganic salts, such as sulfates or carbonates, the viscosity of the polymers could be considerably reduced.

SUMMARY

Observations are made on the effect of polymer flocculants on raw melt liquor and clarifier scum. The materials used are process materials freshly collected from the refinery flows. Each series of tests is carried out uniformly for the purpose of comparison.

Among those polymers evaluated, the leading ones are grouped. It is interesting to note that, in order to show the same activity on raw melt liquor, the dosages of three polymers varied from 8 p/m to 80 p/m. The leading polymers for raw melt liquor are also the leading ones for scum flocculation. They are all anionics. The cationics and nonionics do not compare with these anionics.

The filtration tests on raw melt liquor showed reversed effect from those on scum. In the melt liquor, the polymers retard filtration rate. The bridging effect in scum is not the same as that in cane juice mud.

High-molecular-weight polymers may give high viscosity in solution, and may be difficult to dissolve. However, the best performing polymer observed has excellent solubility. It has also been learned that the polymers may be formulated to reduce their viscosity.

DISCUSSION

A. B. RIZZUTO (Amstar): Would you care to comment on the specificity of the polymers with respect to gums and waxes as opposed to suspended solids?

J. C. P. CHEN: No, I did not go into it that far. I just showed how to use the different types of polymers with the process materials. I do not have anything to show their specific functions.

A. B. RIZZUTO: Would you care to give us the generic name for No. 3?

J. C. P. CHEN: It is Percol LT-25, by Allied Colloids.

P. PETRI (Godchaux-Henderson Sugar): Have any of the cationic polyelectrolytes that you use been approved by the Food and Drug Administration?

J. C. P. CHEN: I don't think the cationics used in my test have been approved yet for sugar liquor. My intention was to find out if they were really worthwhile at all, and later to go ahead and find out whether they can get clearance.

P. H. PETRI: How will you go about obtaining the approval of the FDA?

J. C. P. CHEN: That would be up to the manufacturers of the polymers. The cationics used in my tests are not comparable to the anionics.

P. PETRI: Well, I am familiar with some of the cationic additives, and it seems that the FDA approves them only if they are below levels of 10 p/m for potable water. However, they seem to be very effective at levels of 150 p/m, when added to raw liquor treated with phosphoric acid and lime, and at these levels we have achieved decolorization of up to 45%. The problem is this: It is possible to determine the residual amounts of the polyelectrolyte in, say, clarified liquor, but the FDA requires that the same thing be done with the refined product. It is very difficult in the laboratory to duplicate the crystal that you produce in a big vacuum pan, and unless you do that, you have no way of submitting a sample of your finished product to the FDA for examination. Of course, you can't afford to run the additive through the refinery without FDA approval, and I am wondering how you would go about solving that problem—you would have that in the raw mills too.

J. C. P. CHEN: According to the Federal Register, Subpart D, Food Additive, §121.1092, the limit is on 5 p/m by weight of juice (about 13% solid). If this is applied in refinery liquor, and based on solids or raw sugar melted, it would be equivalent to 40 p/m by weight of solid. In order to be able to submit a sample (or samples) to the FDA for examination, the only way is to run a pilot-scale pan and check the crystal and runoff. I suppose this was what those polymer manufacturers did to submit raw sugar samples for examination.

S. STACHENKO (Redpath): I wasn't quite clear on the sequence of events in the addition of flocculants. My understanding is that you take your melter liquor and add the flocculant. Now, what is the actual sequence of events in your purification scheme in the refinery? What do you do after that?

J. C. P. CHEN: The liquor for our flocculation tests was collected from our regular, treated, melt liquor flow, before it entered a clarifier.

S. STACHENKO: Do you add phosphoric acid?

J. C. P. CHEN: The liquor was already heated and treated with phosphoric acid and lime. It was the regular process stream going to the clarifiers. The liquor collected for tests was after the phosphoric acid and lime treatment and before air was added.

F. M. Williams (Pittsburgh Activated Carbon): Does No. 3 have FDA clearance?

J. C. P. CHEN: I haven't seen the clearance in writing. Recently I have checked again with the polymer manufacturers and they expect to obtain a written confirmation soon.

T. M. PEARSON (Imperial Sugar): Could you comment on the relative cost of Nos. 1, 2, and 3?

J. C. P. CHEN: I do not have the exact prices, but No. 2 is about half the price of No. 1, and No. 3 is about the same as No. 1.

GAS-LIQUID CHROMATOGRAPHY OF MINOR CONSTITUENTS IN SUGARS

By Mary A. Godshall[1]

ABSTRACT

Progress in solving the problems related to measurement of minor constituents in sugars by gas-liquid chromatography (GLC) is reported. The optimum GLC conditions were determined and methods of obtaining the minor constituents free of the great excess of sucrose investigated. Four constituents—malic acid, *p*-hydroxybenzoic acid, palmitic acid, and oleic acid—were followed throughout the refining process. All were in the part-per-million range in raw sugar and 10 to 100 times less in refined sugar.

INTRODUCTION

The sugar industry has always been interested in color, particularly in what it is and how to get it out. These important questions may also encompass all nonsugar constituents of sugar whether they are colored or not. In this era of intense concern for environmental health, the existence of minor constituents in sugar takes on an added dimension.

Much progress has been made in the identification of natural plant pigments that appear in raw and refined sugar (2-4).[2] Work has also been done on the identification of volatile aroma components (*8*).

Once the identity of some of the minor constituents is known, the next obvious step is to devise a method of measuring the amount of these materials in the sugar. Since they are evidently present in very small quantities, one effective technique that could be used is gas chromatography, which is well known to be a sensitive analytical procedure. Gas chromatography has already been used in the sugar industry for the quantitative measurement of aconitic acid (*6*) and sugars (*7*), as well as in the beet sugar industry (*5*).

Essentially four problems must be overcome in order to use gas-liquid chromatography (GLC) successfully in the measurement of minor constituents in sugars: (1) The constituents must initially be separated from the sucrose and invert sugars which would otherwise dominate the chromatogram; (2) suitable chromatographic conditions that will allow good separation of component peaks in the shortest length of time must be determined; (3) the peaks in the chromatogram must be identified; and (4) a procedure must be developed for measuring them. This report describes our progress in solving these problems.

MATERIALS AND METHODS

Extraction of Constituents

Extraction of the minor components from the sugar depends upon a solvent in which sucrose and invert are not soluble, but in which only some constituents are soluble. If too many compounds are extracted, the crowded chromatogram that results would be useless for peak identification. Another desirable quality of the solvent is immiscibility with water so that sugar liquors can be studied without the problem of water removal.

An investigation into the extraction capabilities of a number of common organic solvents showed that all solvents extracted a large number of different compounds from raw sugar. However, solvents that were similar in structure (i.e., alcohols or esters) produced very similar chromatograms with the most obvious differences being in relative amounts of individual components extracted. Dissimilar solvents resulted in entirely unlike chromatographic patterns.

Although the low-molecular-weight alcohols

[1] Chemist, Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, New Orleans, La. 70179.

[2] Italic numbers in parentheses refer to items under "References" at the end of this paper.

are soluble in water, a study of various alcohol extracts of a solid raw sugar (not a solution of raw sugar) was done. In all cases, the alcohols extracted so much sucrose and invert that these peaks dominated the chromatogram. Figure 1 shows the chromatogram of an ethanol extract of a solid Florida raw. It demonstrates quite well the unsuitability of low-molecular-weight alcohols for extraction of minor constituents.

Figure 2 is a chloroform extract of the same sugar, also extracted in solid form. In this chromatogram, there are too many peaks too close together, making identification of individual peaks difficult and uncertain. Benzene, like chloroform, while extracting no discernible sucrose or invert, extracts too many components.

It is interesting to note that most solvents extract compounds that elute in the first half of the GLC run, or in the first 15 min, whereas petroleum ether extracts compounds that elute after 15 min. Figure 3 shows the petroleum ether extract of solid Florida raw. Peak 1 was identified as palmitic acid and peak 2 as oleic acid.

Ethyl acetate fulfilled all the necessary requirements for a solvent and was therefore chosen for the experiments. In addition, much of the preliminary work on identification of plant pigments done in this laboratory had been done with ethyl acetate extracts (*3*).

Constituents were extracted from the sugar by shaking five times a pH 6–7 solution of sugar in a separatory funnel with 50-ml portions of ethyl acetate. Raw and refined sugars were extracted at 25 Brix, whereas refinery liquors were extracted without any adjustment of Brix. A satisfactory amount of extract was obtained from 50 g of raw sugar, 150 g of refined sugar, and approximately 100 g of solids in liquors.

After extraction was completed, the 50-ml portions of extract were combined, and water was removed by the addition of anhydrous sodium sulfate. After drying, the extract was filtered, concentrated to about 1 ml, and transferred to a silylating vial; the remaining solvent was removed under vacuum. The extract was then redissolved in a commercial silylating agent, Tri-Sil,[3] to which the internal standard had already

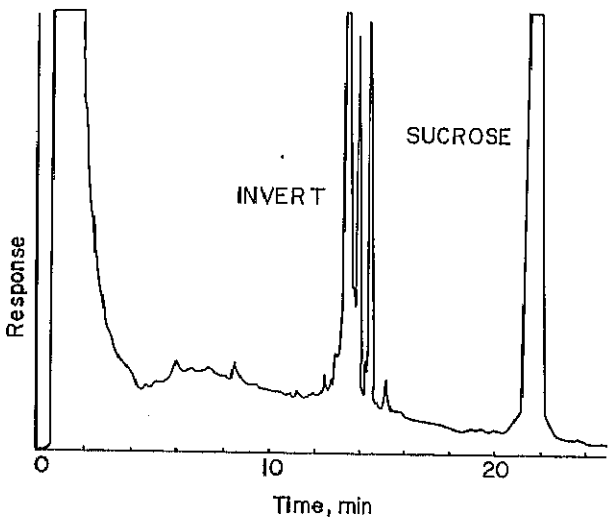

FIGURE 1.—Chromatogram of ethanol extract of solid Florida raw sugar.

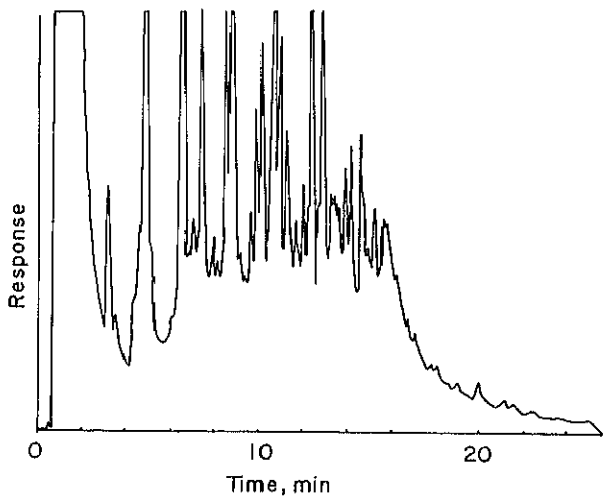

FIGURE 2.—Chromatogram of chloroform extract of solid Florida raw sugar.

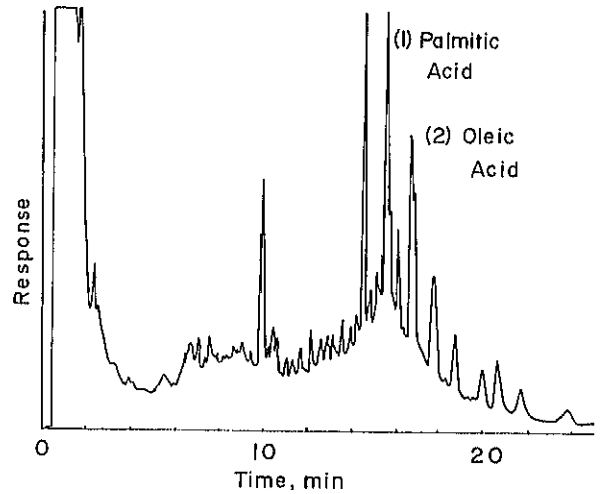

FIGURE 3.—Chromatogram of petroleum ether extract of solid Florida raw sugar.

[3] Pearce Chemical Company.

been added. At this time the extract was ready for gas chromatography and measurement.

It should be noted that the measurement was made on the amount *extracted* by the above procedure. Any incompleteness of extraction was not taken into account. However, since the same procedure was used for all this work, the results should be internally comparable, and only slightly lower (by the degree of extraction) than the true values of the amount of the *sugar*.

GLC Conditions

Along with developing an extraction procedure, it was necessary to develop gas chromatographic conditions that would give well-resolved peaks in the shortest possible time.

Gas chromatography was carried out with a model 5750 Hewlett Packard research chromatograph fitted with dual-flame ionization detectors and 6-ft by 1/8-in (external diameter) stainless-steel columns packed with 1.5% silicone gum rubber (SE 30) on 80/100 mesh Chromosorb HP. The SE 30 column was chosen because it is one of the best for general purpose work when there are many different types of compounds being studied, as it is nonselective, separating on the basis of molecular weight. It is also temperature stable up to 300°–350° C.

After much experimentation, the program that gave the most satisfactory results was the following: 4-min postinjection period at 100° C; temperature increase of 15° C/min to 270° C; hold for 10 min at 270° C and recycle. The carrier gas was helium with a flow rate of 17 ml/min. The temperature of the injection port and detector was 300° C. These conditions resulted in sharp, well-resolved peaks with a minimum of tailing and a recycle time of less than 30 min, thus allowing quite a few determinations in one day.

Identification of Peaks

Peaks were identified by comparing retention time of peaks in the extract with that of constituents known to be in sugar and extractable by ethyl acetate. Confirmation was done by adding the known compound to the extract and running the "spiked" extract to see if there was enhancement of the suspected peak.

Four major peaks in an ethyl acetate extract of raw sugar were identified and these were measured throughout various refining steps, as well as in refined sugars. The four constituents

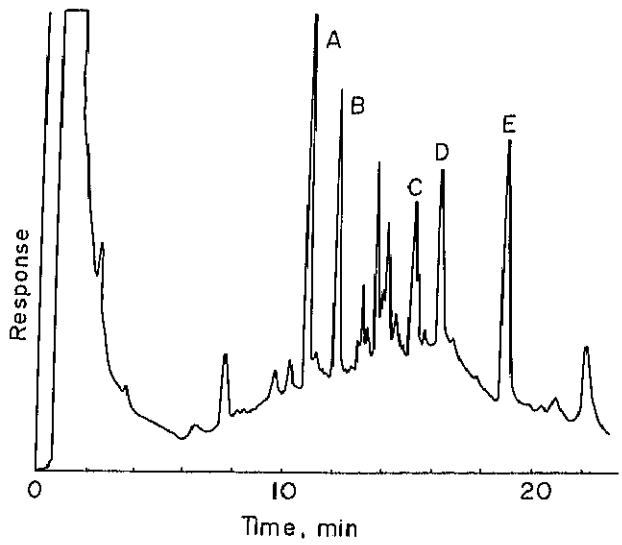

FIGURE 4.—Chromatogram of ethyl acetate extract of Argentine raw sugar solution. A=malic acid, 13.6 p/m; B=*p*-hydroxybenzoic acid, 6.9 p/m; C=palmitic acid, 13.4 p/m; D=oleic acid, 24.2 p/m; E=TPE.

are malic acid, *p*-hydroxybenzoic acid, palmitic acid, and oleic acid. This chromatogram is reproduced in figure 4. These constituents were not always major constituents in the various stages of refining that were investigated.

The presence of palmitic acid and oleic acid as major components was surprising. However, Balch (*1*) in 1947 reported that these two acids, along with stearic acid, were the major acid fractions in the makeup of crude sugarcane wax. Further confirmation was done by making petroleum ether extracts of both solid raw sugar and aqueous raw sugar. As expected, these acids were selectively extracted by this solvent, representing the largest fraction of the extract in both cases (fig. 3).

Measurement of Constituents

The internal standard method of measurement was chosen as the one best suited to the present study. This method has the advantage of eliminating concern about a great many GLC variables, such as flow rate fluctuations and amount injected onto the column, which would otherwise have to be considered in the calculations. It is, instead, assumed that the standard will be affected in the same manner as the compounds being measured. Therefore, the response of the constituent is related to the response of the standard, and the resulting ratio (K) is a constant that

can be used to determine how much of the constituent is in any given extract.

The choice of the standard depends upon three qualities: (1) It cannot elute where it would interfere with any of the constituents in the chromatogram; (2) it must be easily and completely soluble in the silylating solvent, Tri-Sil; and (3) it preferably should not have to be silylated in order not to use up any of the Tri-Sil. Tetraphenylethane (TPE) was the standard used. Its elution time is approximately 18 min, past the point where all constituents are eluted and before sucrose is eluted, at about 22 min.

The relative response value, or K-value, of the constituents in relation to the standard, TPE, was calculated by preparing samples of known weights of the standard and each of the four constituents. Peak heights were carefully measurd and used in the following formula:

$$K = \frac{\text{peak height of constituent} \times \text{weight of internal standard}}{\text{peak height of internal standard} \times \text{weight of constituent}}$$

Peak height was used instead of the more commonly used peak area because of the extreme narrowness of the peaks in the chromatograms. At times, also, the peaks have shoulders or small, unresolved side peaks that would affect the area but which do not contribute to the height of the main peak.

The recorder used for this work has a two-pen setup, in which both pens simultaneously record the output, but one pen does so at only one-tenth the sensitivity of the other pen. If any peak should go off scale on the more sensitive pen, the peak height can easily be determined by multiplying the height of the one-tenth duplicate peak by 10. The duplicate set of smaller peaks is not reproduced in any of the figures given here because it would obscure the chromatogram.

Table 1 gives the K-values that were obtained. In the case of oleic acid, the response varies according to peak height in a direct linear relationship, as shown in the diagram in figure 5. In spite of the variable K-value, the results of oleic acid were fairly reproducible, as demonstrated in table 2. These represent the data obtained for both a raw sugar and a refined sugar.

TABLE 1.—*Relative response factors*

Constituent	K-value
Malic acid	2.37
p-Hydroxybenzoic acid	2.42
Palmitic acid	.75
Oleic acid	(1)

[1] Variable.

Once the response values of the constituents were known, measurement consisted of preparing extracts containing a known weight of the standard and using the following formula to calculate the weight of the compound in the extract:

$$\text{Weight of constituent} = \frac{\text{peak height of constituent} \times \text{weight of standard}}{K\text{-value} \times \text{peak height standard}}$$

The result is then converted into parts per million.

To get a known weight of standard into the extract, a solution of TPE and Tri-Sil was prepared. An aliquot of this solution was used to silylate each extract and at the same time to deliver a known weight of the TPE to the extract.

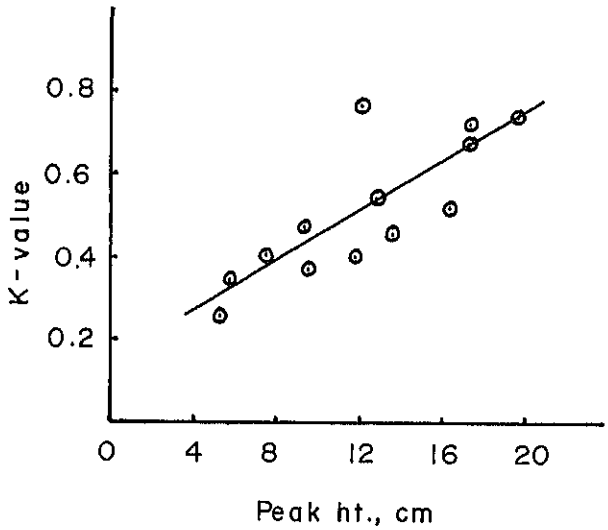

FIGURE 5.—Relative response of oleic acid.

RESULTS

Several representative chromatograms of ethyl acetate extracts are reproduced in figures 4, 6, and 7. Figure 4 shows the extract of 50 g of 25-Brix Argentine raw sugar. In this particular raw, the major ethyl acetate extractable components are the four compounds under investigation. The amounts ranged from 6.9 p/m for p-hydroxybenzoic acid to 24.2 p/m for oleic acid.

TABLE 2.—*Precision of results for oleic acid, parts per million*

Raw	Refined
24.2	0.57
21.0	.65
28.8	.69

Figure 6 shows the extract of a 62.5-Brix clarified refinery liquor that had been filtered once through partially spent bone char and once through resin. This extract illustrates that the four major constituents in the raw are not necessarily major constituents in other refinery liquors. The amounts of components measured are less than 1 p/m at this stage.

Figure 7 shows the extract of a 25-Brix solution of refined sugar. Again, the picture is quite different. Most of the compounds eluted early have been eliminated or are below measurable limits, leaving a series of compounds that are eluted between 14 and 17 min, including palmitic and oleic acids.

Table 3 summarizes the results obtained for various raw and refined sugars. The numbers represent the mean values and are given in parts

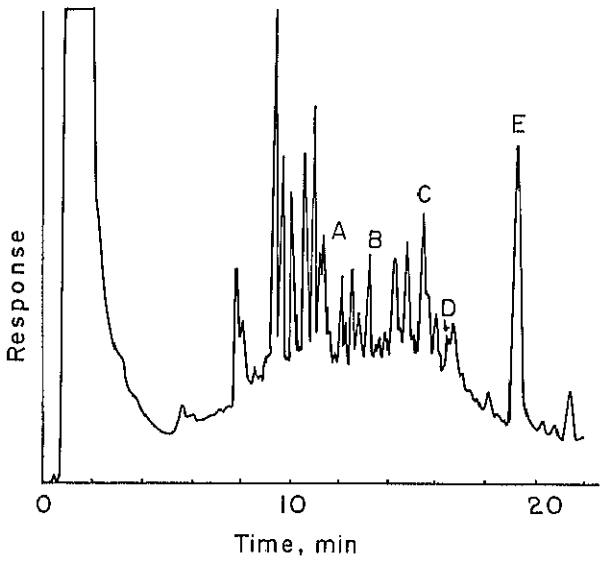

FIGURE 6.—Chromatogram of ethyl acetate extract of clarified washed sugar liquor filtered once through partially spent bone char and once through resin. A=malic acid, 0.12 p/m; B=*p*-hydroxybenzoic acid, 0.085 p/m; C=palmitic acid, 0.43 p/m; D=oleic acid, 0.41 p/m; E=TPE.

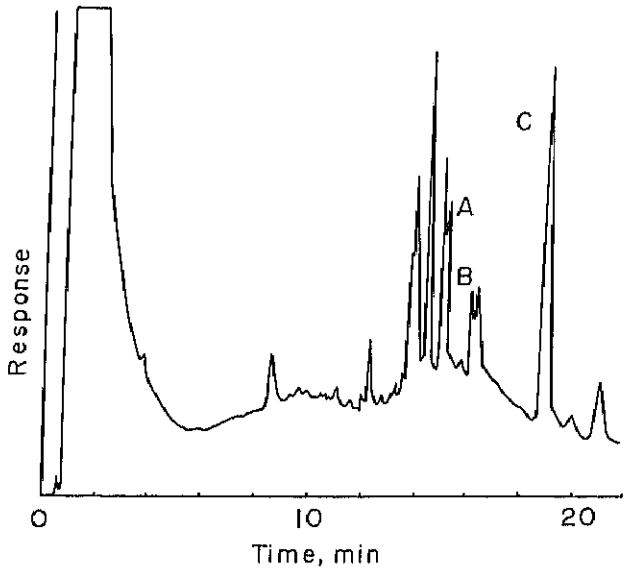

FIGURE 7.—Chromatogram of ethyl acetate extract of refined sugar solution. A=palmitic acid, 0.3 p/m; B=oleic acid, 0.39 p/m; C=TPE.

TABLE 3.—*Minor constituents in ethyl acetate extracts of some raw and refined sugars, parts per million*[1]

Sugar	Malic acid	*p*-Hydroxybenzoic acid	Palmitic acid	Oleic acid
Argentine raw (25 Brix)	13.3	6.8	12.1	24.7
Australian raw (50 Brix)	.59	.93	3.8	6.6
Washed raw	.23	.12	.90	1.69
Minimally refined	.12	*.004*	.54	.64
Refined by—				
Carbonatation	*.012*	*.007*	.33	.37
Phosphatation	*.002*	*.006*	.31	.36

[1] Values printed in italic are below the limit of detection and are approximations.

per million to afford a basis of comparison of all extracts. The numbers obtained give some idea of the range that is possible between raw sugars. Refined sugars show much less variation. The last two refined sugars are from the same refinery, with one having undergone clarification by carbonatation and the other by phosphatation. As can be seen, the results for the four compounds studied are quite similar for each sugar. The chromatograms are also essentially the same.

TABLE 4.—*Minor constituents in ethyl acetate extracts of liquors from one refinery, parts per million*[1]

Sugar	Malic acid	*p*-Hydroxybenzoic acid	Palmitic acid	Oleic acid
Washed sugar to clarifier	0.23	0.12	0.90	1.69
Clarifier to char	.051	.058	.50	.66
Once over partially spent char	.13	.11	.41	.43
Once over partially spent char and once over resin	.12	.083	.44	.44
Once over partially spent char and once over freshly regenerated char	.045	.088	.35	.45

[1] This refinery used phosphate clarification, bone char, and IRA 901 ion exchanger in the chloride cycle.

Table 4 shows the results obtained for extracts of process liquors from one refinery, each at a different stage of refining. In general, malic and *p*-hydroxybenzoic acids behave alike, the oleic and palmitic acids behave alike. Some interesting observations about the relative efficacy of char and resin can also be made.

In all cases, palmitic and oelic acids both show a steady decrease in the amount present as refining proceeds. Palmitic acid content drops after phosphate clarification and after one filtration through partially spent char. It remains essentially the same with filtration through resin, but does drop slightly after the second filtration through freshly regenerated char. Therefore, it would appear that char alone effectively removes palmitic acid, with resin evidently not contributing to its removal.

The oleic acid level drops considerably after clarification. It drops again after one filtration through partially spent char and remains unchanged when filtered again through either char or resin. As was the case with palmitic acid, char effectively removes oleic acid, with resin apparently not contributing to its removal.

Malic acid and *p*-hydroxybenzoic acid, unlike the fatty acids, do not exhibit the steady decrease in quantity nor the same type of behavior on char. These acids both drop dramatically after phosphate clarification. Both increase just as dramatically after passing through the partially spent char, malic acid increasing up to one-half its original amount and *p*-hydroxybenzoic acid up to the original amount. Since this is a partially spent char, it is possible that these acids are being desorbed off the char.

After the liquor that has been through char once is filtered through resin, the quantity of malic acid remains unchanged. However, when the liquor is filtered the second time through freshly regenerated char, the level drops to that of liquor after clarification. This would certainly indicate that although partially spent char is incapable of malic acid removal, a regenerated char is effective. Resin appears to have no effect in the removal of malic acid.

In the case of *p*-hydroxybenzoic acid, the amount present drops after the second filtration through either char or resin. Resin and char that are freshly regenerated appear to be equally effective in the removal of this acid.

THE EFFECT OF BRIX

During the course of this investigation, it was discovered that Brix affects the amount extracted by ethyl acetate. Table 5 shows the amount of each component extracted from two raw sugars at different Brix. Since solid sugars (raws and refined sugars) were dissolved at 25 Brix for extraction, and liquors were extracted as received, usually in the 60- to 65-Brix range, this is an important variable. Two samples of 25-Brix Argentine raw are included to give some idea of the variation that can exist between extractions.

TABLE 5.—*The effect of Brix on the results of ethyl acetate extracts of two raw sugars, parts per million*

Sugar and Brix	Malic acid	*p*-Hydroxybenzoic acid	Palmitic acid	Oleic acid
Argentine raw:				
25 Brix	13.3	6.8	12.1	24.7
25 Brix	15.4	7.4	14.8	17.8
65 Brix	27.4	4.7	4.5	6.2
Australian raw:				
25 Brix	.30	.76	4.7	9.0
50 Brix	.59	.93	3.8	6.6

In both raws, the extractability of malic acid is increased at higher Brix; it is essentially doubled, whereas high Brix would appear to considerably inhibit the extractability of palmitic and oleic acids. The extraction of *p*-hydroxybenzoic acid is not affected as much by Brix. It is also evident that Brix is not as great a factor in a higher quality raw sugar such as the Australian raw, where the range of variation in quantities at 25 and 50 Brix is not as great as in the Argentine raw, except in the case of malic acid, where the level doubles at the higher Brix, as it did in the Argentine raw.

CONCLUSION

This concludes the work done up to the present. There are still problems to be worked out, especially on concentration effects and completeness of extraction. The results so far, however, have indicated that the method is workable, with a fair degree of reproducibility, and is applicable to many of the minor constituents in sugar.

REFERENCES

(1) Balch, Royal T. 1947. Wax and fatty byproducts from sugarcane. Tech. Rep. No. 3, p. 28. Sugar Research Foundation, Inc., New York.

(2) Farber, L., and Carpenter, F. G. 1971. Chlorogenic and caffeic acids identified as colorants in cane sugar. Int. Sugar J. 73: 99.

(3) ———. 1972. Identification of cane pigments that persist into refined sugar. Proc. Int. Soc. Sugar Cane Technol. XIV Congr., pp. 1589–1600.

(4) Gross, D., and Coombs, J. 1971. Chlorogenic and caffeic acids in sugarcane. Int. Sugar J. 73: 100.

(5) Kiely, M., and O'Drisceoil, P. 1971. Lactic and volatile acids in beet sugar manufacture. Part I. Lactic and volatile acids in molasses. Int. Sugar J. 73: 135–136.

(6) Mehltretter, C. L., and Otten, J. G. 1971. Aconitic acid determination by gas-liquid chromatography. Int. Sugar J. 73: 235.

(7) Velasco, V. S., Heisler, M., and Dowling, J. F. 1970. Quantitative measurement of sugars by gas-liquid chromatography. Proc. 1970 Tech. Sess. Cane Sugar Refining Res., pp. 61–81.

(8) Yokota, M., and Fagerson, I. S. 1971. The major volatile components of cane molasses. J. Food Sci. 36: 1091–1095.

DISCUSSION

W. W. BINKLEY (New York Sugar Trade Laboratory): Referring to table 5, it seems to me that you might account for the lower palmitic and oleic acids in the higher Brix solutions simply through a stripping effect. These acids can be stripped out under vacuum distillation.

N. H. SMITH (California and Hawaiian): When you use chloroform as the solvent, you find too many peaks. Can you not either vary GC conditions or use another solvent on that extract to get a more meaningful chromatogram?

M. A. GODSHALL: Yes, I'm sure we could, but we haven't tried that yet.

V. S. VELASCO (CPC International): How do you arrive at a base line? It seems to me that it isn't straight.

M. A. GODSHALL: The base line is a result of the system we are using. We use pyridine as the solvent that contains the silylating agent, and with the high rate of temperature increase that we use, pyridine evidently absorbs onto the column and is then driven off as the temperature increases. The base line was obtained by running the Tri-Sil by itself and then assuming that the peaks that come out were eluted just above the base line.

V. S. VELASCO: How much raw sugar do you use for extraction to get a measurable amount of minor constituents on the gas chromatograph?

M. A. GODSHALL: We use 50 g of the raw, 150 g of the refined, and about 100 g of the liquors.

S. STACHENKO (Redpath): You indicated in table 4 that the level of malic acid had gone up after charring. Would you not expect this, because liquor going over char at a relatively high pH would probably react in terms of invert and produce some organic acids anyhow?

F. G. CARPENTER (Agricultural Research Service): I think that this is a prime example of taking one step forward and two steps backward. That was once-used char. It was a double filtration in this particular operation, and the char had caught the malic acid very nicely the first time and then was putting it back on in the second pass. In the same table 4, the use of new char actually cuts down the malic acid.

B. A. SMITH (USDA): Could you tell us what happened to the aconitic acid in these sugars?

M. A. GODSHALL: The system that we are using is to extract neutral solutions with ethyl acetate, and the ethyl acetate does not take the

aconitic acid out. If you acidify the solution, then you do extract the aconitic acid. We really did not want to be worried with aconitic acid, since there are other methods of determination already available.

M. C. BENNETT (Tate & Lyle): It's a mammoth piece of work you are undertaking, and I think those of us who have had any connection with it understand just how difficult it is to attach names to those peaks. Some of the things of interest to our company are odor and flavor, particularly in soft yellow sugars and sirups, which we are selling to confectionery companies. That all involves taste panels and organoleptic tests. I would be interested if you could say something about what you think you can do in this area with those chromatograms. Do you see a pattern emerging as part of your program of research work?

F. G. CARPENTER: Some of those peaks certainly are from odors and taste components. As Mrs. Godshall pointed out for aconitic acid, if you change the pH, you get a whole new set of peaks, so there are even more than appear here. We have a good chromatographer here now, and I think she has done very well in identifying four components already with considerable certainty. One by one we will find out what some of those other peaks are. We've got a lot of components already named; we need a little more time to correlate these with peak positions. Hopefully, some day we will find out what peaks are the molasses flavor, and what peaks are the molasses odor, and we will be able to pinpoint the properties given by specific constituents.

G. W. MULLER (Kerr-McGee): Are the colorants part of these multiples of peaks?

M. A. GODSHALL: When you talk about brown colors, you are getting into high-molecular-weight compounds, and gas chromatography is not the tool of choice. Some of our extracts are highly colored whereas others are not, and yet both types give essentially similar chromatograms. This is dramatically evident in our alcoholic extracts of raw sugars. These extracts, especially the methanolic extracts, are quite dark, but on chromatography, no peaks attributable to this color are present. We therefore feel that the color itself is not going through the column.

F. G. CARPENTER: I might add that only one of the colored compounds we have identified[1] showed up. There are lots of things in there beside color—look at those lists of constituents that have been found in sugar already. There are many carboxylic and amino acids present, which are not colored, but do show up on the gas chomatograph. If we can change solvents and gas chromatographic conditions, then maybe we can get rid of some of the already known constituents and find the colored ones. Again we are using "color" in the broad sense of which I spoke of yesterday. "Colorants" to me means all minor constituents.

[1] Farber, L., and Carpenter, F. G. 1972. Identification of cane pigments that persist into refined sugar. Proc. Int. Soc. Sugar Cane Technol. XIV Congr., pp. 1589–1600.

GENERAL DISCUSSION

W. W. BINKLEY (New York Sugar Trade Laboratory): I would like to comment about the problem of a standard invert solution for standardizing the copper reagent for the determination of reducing sugars in sugar products. For a long time, we have assumed that, if we simply invert sucrose with dilute acid, we get exactly equal amounts of glucose and fructose. This conclusion was based more on hope than on actuality. Now that we have sophisticated instruments, it is quite clear that we do not get from sucrose, under those conditions of acid inversion, equal amounts of glucose and fructose. There is always less fructose than glucose. The more drastic the conditions for the inversion, then the less fructose we can expect to get. The effects of acid may be avoided in part by using invertase to effect the inversion.

My present solution is to mix analytically pure glucose and analytically pure fructose in equal amounts and use that for a reference standard.

C. J. Novotny (Industrial Filters): Two years ago in Boston we talked about some work we had done using ion exclusion on molasses. Some of the people at this meeting remembered that, and asked what we had done since, so I will talk about our recent developments in that field. We found that working in our lab facilities was rather difficult because we had problems with transporting drums of molasses to Cicero, and finding somebody qualified to analyze our data and results. SuCrest in Chicago was very cooperative but we did not want to outlive our welcome so we took an alternate course.

We have been cooperating with a German beet sugar company, Pfeifer and Langen, at their factory at Euskirchen. They became quite interested in the process, and have progressed beyond the laboratory stage; they are now in phase 2, in a pilot plant. We had hoped that the pilot plant would have been operating for several months now, but as many of these projects go, it is not, so we do not really have any results to report. However, we did cooperate in the design of a column which might be of interest.

From the standpoint of product per volume of resin, ion exclusion is really rather poor; it takes a lot of resin to get much product at all. So, one of the things we tried to do in conjunction with Pfeifer and Langen was to operate with as deep beds as possible because the deeper the bed the narrower the diameter, or the fewer the columns for the same amount of resin. This introduced another problem: In running 60 Brix through a deep bed, the pressure drop markedly increases. The problem is compounded with the compaction of the resin due to changing volume. The result is that the pressure drop multiplies again.

In the ion exclusion process, if you are treating a heavy sirup, you might be running something as heavy as 60 Brix through the column. Then you will be following that with the recycled fractions which may be 20 Brix or so and then you will follow that with water, and so the resin itself changes in volume quite a bit. It has been found by Mountfort[1] and others that the lower cross-linked cation resins are best for ion exclusion. Unfortunately, they are the worst from the standpoint of physical stability. As the volume of the resin changes, the resin grows in a vertical direction, of course, but it also tries to grow in a horizontal direction, and that means quite a bit of internal pressure in the column. Being rather physically weak resins, they tend to crush, and this is an expensive operating cost. To try to eliminate this problem we built the column as in figure 1.

Basically, this is a cylindrical column. The particular one operating in Germany now is 10 meters tall, with about a 5½-meter bed depth, so it is in the 17- to 18-foot range resin bed. The flow rate is about eight-tenths of a bed volume per hour (B.V.H.), and we hope to get to 1 or 1.1 B.V.H.

We also have found that as the bed compacts, ion exclusion becomes much poorer. This particular column is built with the center section like a rubber hose—just a hollow core built of a flexible material, with a dome connected to the inside and separated from the rest of the inside of the column, as in figure 1. As the bed expands, it

[1] Mountfort, C. B. 1965. Purifying solutions containing sugars. U.S. Patent 3,214,293.

tends to expand vertically and horizontally. It is desirable to have it not expand vertically because then you have a distribution problem, with the inlet either 3 feet above the bed or 6 inches into it, since with a bed this deep in a conventional column there can be more than 3 feet expansion involved. So, as the bed expands, it partially collapses that rubber core instead of growing upwards and so stays relatively level. This method also prevents the bed from crushing itself. When you run liquor through, and the bed shrinks back down, a counterpressure in the top of the dome refills the hollow core (which is filled with water in this case) and keeps the bed level again, thus keeping the pressure drops in order.

About 2 weeks ago, as usual, complications developed. The hose broke, and the level control was

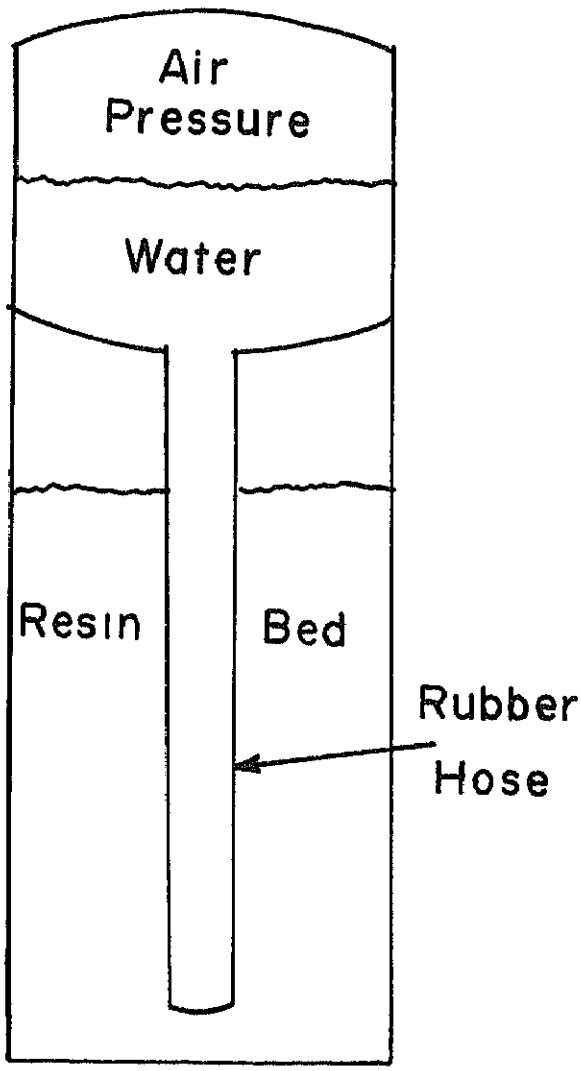

FIGURE 1.—Device to compensate for bed expansion.

no longer possible. The problem, we decided, was that we had attempted to make the hose out of an FDA-approved rubber, but the one that was readily available in Germany did not have good self-adhesive properties. A long flexible hose such as this tends to sag when the temperature is fairly high, about 85° C. So, if we just hung it in the column, it would collapse with heat application. It was therefore necessary to put a reinforcement of textile between two layers of rubber. But the layers did not bond together very well, and delaminated after several weeks in the column. So we are going to make a new core of a different rubber.

We also had the problem of a fluctuating level, so to compensate for that while we built a new hose, we made a stainless-steel float with a rod guide through the center. We adjusted the amount of water that we put into this float so that it floated on the top of the resin, but not in the juice. Then we connected an air supply to it with a flexible tube, with an outlet from the float. In effect, we made a bubble pipe as in figure 2. The air put in the float bubbled out just above the bed, regardless of bed location. By measuring the back pressure, on air that came in, we had an idea of the height of the liquid above the resin. As the resin bed expanded and contracted, this float moved with it; it was always measuring the liquid level above the resin. We could still maintain a liquid level of 3 centimeters above the resin bed, even when the bed level itself was varying about 3 feet. We used this to signal a modulating valve on the inlet and we could control the flow based on the bubble pipe estimate.

D. E. TIPPENS (Amstar): Is the hose in your column closed at the top? Is it a pneumatic pressure vessel?

C. J. NOVOTNY: Yes, it is a pressure vessel in itself. It is closed at the bottom and the top is connected to a source of air.

D. E. TIPPENS: If you have a constant air pressure within the hose, would not the difference in hydrostatic pressure up and down the column cause the bottom of the hose to collapse, and the top to expand and thus defeat your purpose?

C. J. NOVOTNY: No, I do not think so. We are not sure, of course, what the shape of that hose is during the cycle itself, because we just cannot see inside the resin bed. However, at any time during the cycle there are several liquids passing through that column. An ion-exclusion cycle lasts

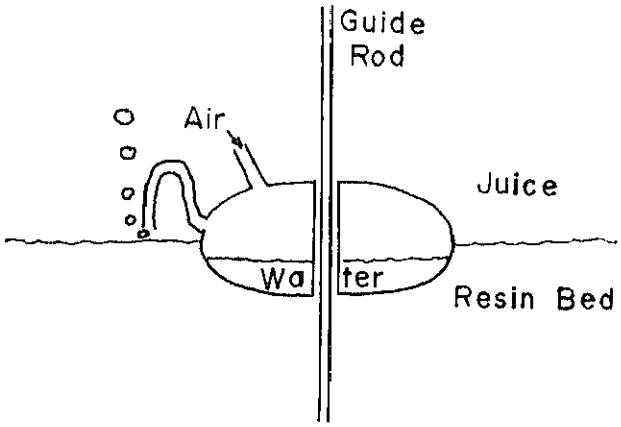

FIGURE 2.—Resin level detector.

about 1 hour, and during that time about eight-tenths of a bed volume of total liquid passes through. Perhaps five one-hundreths of a bed volume of that is the juice being fed, and perhaps half of a bed volume is water, and the other, slightly less than half, is either one or two recycle fractions. So, we have zero-Brix, 60-Brix, and a couple of 20-Brix liquors running through at all times. We do not know what that hose is shaped like at any time, but we do know that it takes the shape necessary to keep the bed volume about right, and the pressure drop in order.

D. E. TIPPENS: At any rate, as long as the hose does not collapse entirely at the bottom or elsewhere, the resin bed level should hold constant.

C. J. NOVOTNY: Yes, that is right. I doubt if it would collapse completely because there is a counterpressure—the column of water—applied to it, inside, to prevent that. We have been attempting to run 5 days, 24 hours a day, each week. That was possible for a couple weeks with the rubber hose in there. Since the hose broke we have been operating with the float device and we have noted that after about 3 days the bed packs so much that we have to stop running and backwash it, to loosen up the bed and thus keep our flow rates up. With the hose in, it was not necessary to backwash. We do not know how long it could have gone on without backwashing, because we have to stop on weekends, but it works.

F. M. CHAPMAN (Chapman Associates): With the 5 meters of resin that you use, the simple hydrostatic pressure should be about half an atmosphere. Laterally, the pressure exerted by the resin may be much more than that, but you have plenty of elasticity in the hose.

C. J. NOVOTNY: Yes, there is not much force, but the internal force on the resin beads themselves, I am sure, is tremendously in excess of that force when they expand sideways against the walls of the column.

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